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ANTIFUNGAL ACTIVITY OF POMEGRANATE PEEL EXTRACTS AGAINST *FUSARIUM OXYSPORUM F.SP.RADICIS –LYCOPERSICI*

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ABSTRACT

The objective of our study was the antifungal power test of the pomegranate peel aqueous extract PPE, on a phytopathogenic fungal strain of tomato "*Fusarium oxysporum f.sp.radicis-lycopersici*". For this purpose, a test was carried out, by the direct contact method in PDA potato dextrose agar medium, on the phytopathogen, in different concentrations of PPE (1%, 2%, 3%, 5%). Antifungal activity was evaluated by the estimation of the mycelial growth, the mycelial inhibition rate and the spore count. Our results showed a remarkable and highly significant antifungal activity against the studied phytopathogen, an inhibition index of 100% with the 3% and 5% concentrations was noted. The effectiveness of PPE could be the subject of investigation and exploitation in the integrated control of the tested fungus that causes much damage to the tomato crop.

Key words: *PPE, Fusarium oxysporum f.sp.radicis-lycopersici, mycelial growth, rate inhibition, sporulation.*

INTRODUCTION

Plant pathogens reduce crop yield and quality, whose control in traditional agricultural production systems relies mainly on the application of fungicides (Romanazzi et al.,2012; De Corato et al., 2016), which reduces the stability and sustainability of agricultural production (Larsen et al., 2017; Fones et al., 2020). However, the fungicides application presents a serious risks to human and environmental health, with negative impacts on non-target microorganisms (Sanzani et al.,2010; Gill and Garg , 2014).

Several alternative methods have been proposed to inhibit the growth of several pathogens fungal and achieve crop disease control, especially plant extracts (biosourced products) (Romanazzi et al.,2012; De Corato et al., 2016; Mari et al., 2016; Palou et al., 2016; Van Lenteren et al., 2018). These natural extracts possess a biological antifungal compounds that influence the mycelial growth, the

sporulation rate and the germination, varying from a fungistatic effect to complete inhibition. In laboratory tests, the bioactive compounds of plant extracts applied in their raw state or as a fraction, inhibit partially or totally the fungal growth, affecting the colony development when applied at low concentrations (Mahlo et al., 2010; Castillo et al., 2012; Cerqueira et al., 2016).

In this regard, pomegranate peel extracts PPE (*Punica granatum L., Punicaceae*) have emerged as a very promising source of antifungal substances, for the plant control and food pathogens. The phytochemical screening of different parts of the pomegranate fruit; including peel, arils and seeds revealed a high predominance of polyphenols in the peel part (Orak et al., 2012). This explains their particular use in traditional medicine (Shaygannia et al., 2016). Numerous scientific researchers have demonstrated the therapeutic and antioxidant activity of pomegranate peel extracts (PPE), against many serious diseases; including cancer, inflammation, diabetes, cardiovascular diseases, etc. (Li et al., 2016; Stojanovi et al., 2017; Du et al., 2019).

Fusarium crown and root rot is a soil-borne disease, with the potential to limit productivity in glasshouse and field tomato crops. The causal agent is *Fusarium o*xysporum *f. sp*. *radicis-lycopersici*. Fungicides have limited potential benefit for most *Fusarium* diseases. (Sierotzki and Ulrich, 2003).

The aim of this study was the *in-vitro* evaluation of the antifungal activity of the pomegranate peel extracts PPE, against plant pathogenic fungi: *Fusarium oxysporum f.sp.radicis-lycopersici* by the evaluation of the mycelial growth, the inhibition rate of mycelial growth and the spore count.

MATERIALS AND METHODS

Plant material. The plant material choice was based on the pomegranate peel (*P.granatum Linn*), the pomegranate fruit was collected in the Sidi-Ali-Benyoub locality (Table 1) located at 25 km to the south of the Sidi-Bel-Abbés city, the peel has been authenticated, the reference specimens were sent to the plant taxonomy laboratory ,at the environment department of the natural and life sciences faculty, in Djillali Liabés university of Sidi-Bel-Abbés, for identification and confirmation, where the peel samples were deposited and coded (Kanoun et al.,2014 a; Kanoun et al., 2014 b; Kanoun et al., 2016).

Table 1. Bioclimatic aspects of the study area

Characters	Longitude	Latitude	Altitude	Climate
Sidi Ali Benyoub	00:76408°	35:26978°	1061 m	Semi-arid dry and cold

Plant powder preparation. The fruit was washed with tap water; the peel has been dried in the shade, away from moisture and light, at room temperature for one month on a wooden plate. After drying, they have been crushed in a traditional mortar ,then pulverised with a manual grinder, until a very fine powder was obtained, this latter was stored in the refrigerator at +4°C in a hermetically sealed

container, it will be used later for the studied extract preparation (Kanoun et al., 2014 a; Kanoun et al., 2014 b; Kanoun et al., 2016).

Aqueous extracts preparation (hot). The peel powder weighing 400 g was added to 1000 ml of distilled water and boiled under reflux for 6 hours at a temperature of 200°C at 500 rpm. After 6 hours of agitation, the cooled decoctate was filtered on Whatman paper (N°3) under reduced pressure, the separated marc reated with a second extraction, by mixing 500 ml distilled water with 900 ml of moistened marc, under the same operating conditions as previously; the resulting supernatant was concentrated by a freeze dryer (lyophiliser) of Tel Star type at a temperature of -45°C and a pressure of 4.10^{-2} millibars (Kanoun et al.,2014 a). The different experimental groups in comparison with the control are distributed as follows : Group 1 : control ; Group 2 : PPE1% ; Group 3 : PPE 2% ; Group 4 : PPE 3% ; Group 5 : PPE 5%.

Mycelial growth evaluation. The plant extract was diluted in DMSO (dimethyl sulfoxide) to obtain the concentrations of (0%,1%,2%,3% and 5%). 1ml of each concentration was added to each Petri dish containing PDA medium (potato dextrose agar), a rate of 15 ml per dish was introduced (Jamil et al., 2002; Pande et al., 2011).

After the medium solidification, the inoculation was performed with explants of 5 mm diameter obtained from a 7-day culture (from a fungal suspension adjusted to 9×10^5 spores/ml, with a sterile cutter. These explants are positioned in the Petri dishes centre containing increasing doses of the extract (Kolai et al., 2012; Kanoun et al., 2014 a).

The evaluation of mycelial growth was determined by the Rapilly method (Rapilly, 1968), which consists of measuring the mycelium diameter during seven days, using the following formula: L= D-d/2 (L: mycelial growth, D: colony diameter, d: explant diameter). The mycelial growth means were calculated by the following formula: V (mm/day) = (Ln – Ln-1) / n (V: Mean mycelial growth, Ln, Ln-1,.. were the mycelial growths and n,n-1,n-2..etc: Number of days) (Benzohra et al., 2011; Kanoun et al., 2014 a).

Evaluation of the mycelial growth inhibition level. The results obtained from the mycelial growth estimation were expressed as a percentage (%) of the mycelial growth compared to the control, according to the formula described by Leroux and Credet (Leroux and Credet, 1978). T (%) = (L-I /L) x 100 (T: inhibition rate, L: mycelial growth of the control expressed in cm and I: mycelial growth of the treated fungi expressed in cm) (Kanoun et al.,2014 a).

Sporulation test. The evaluation of sporulation was carried out according to the principle of the method used by Maslouhy (Maslouhy, 1989). This test was performed by washing the entire Petri dish containing the fungus with 10 ml of sterile distilled water, to remove all the spores, then the obtained suspension was transferred in a flask filled with 50 ml sterile distilled water, the number of spores for each sample was counted by the Mallassez cell ,under the optical microscope.

Statistical analysis. The results are expressed as means and their standard error (X±ES). The comparison of means was performed using the t "Student" -test. The differences are considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Mycelial *growth*. According to our results illustrated in (Figure.1), the mycelial growth reaches a maximum of 37 mm in the control group and a minimum of 10 mm in the PPE 5% group at D7. Similarly, in the PPE 3% and PPE 5% groups, mycelial growth did not start until the second day, on the other side, the extracts become more active from a dose of 3% and the mycelial growth in the PPE 5% group was stopped at 10 mm at D4, and remained stable until the end of the experiment (D 7).The mycelial growth kinetics of the control group was clearly superior to that of the experimental groups with the different concentrations of PPE (1%, 2%, 3%, 5%).

According to (Figure.2) , it can be observed that, the mean diameter of mycelium in the control group was 20.21± 12.71 mm, which was higher than the experimental groups PPE 1%, PPE 2%, PPE 3%, PPE 5% with values of (12.42±7.72 mm; 11.07±7.65 mm; 8.85 ± 5.59 mm; 6.78± 4.26 mm) respectively. Our results show that extracts at different concentrations have an inhibitory effect on mycelial evolution.

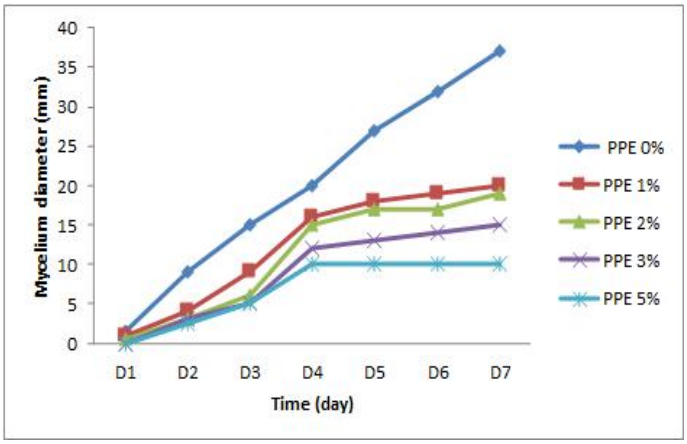

Figure 1. Kinetics of the mycelial diameter evolution according to the different concentration of PPE during 7 days

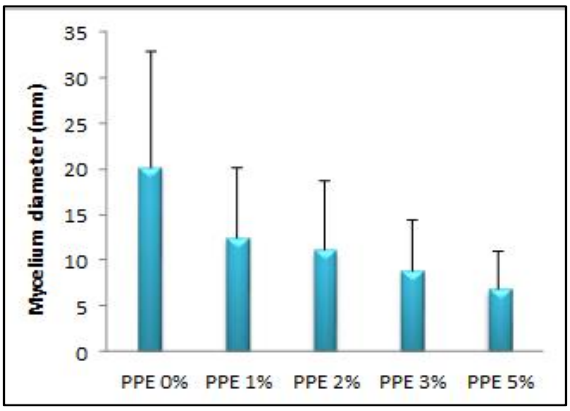

Figure 2. Means myceliums diameters in the experimental groups compared to the control

Inhibition rate of mycelial growth. Our results confirm that both groups (PPE 3%, PPE5%) presented a maximum inhibition rate at D1 which was 100%, while the PPE1% group showed a rate of 20% which was the lowest. It can also be seen that, the inhibition rate kinetics, for all experimental groups was decreasing from D2 to D4, then an increase from D5 to D7 (Figure .3).

Our results confirms the proportional relationship between the PPE dose and the inhibition rate (Figure.4), with a maximum of (70.52± 15.12%) in the PPE 5% group and a minimum of (39±10.98 %) in the PPE1% group.

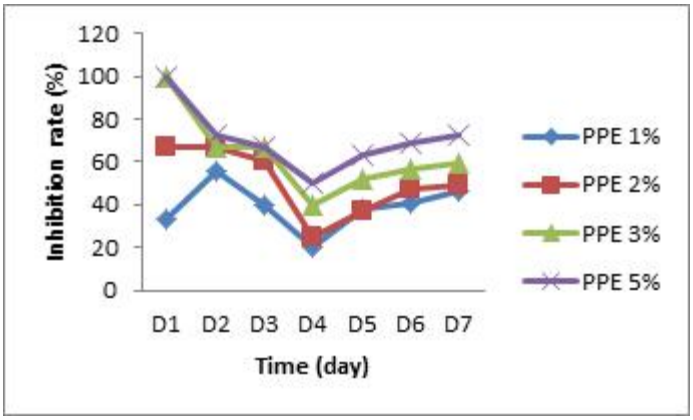

Figure 3. Kinetics of mycelial growth inhibition rate according to the different concentration of PPE during 7 days

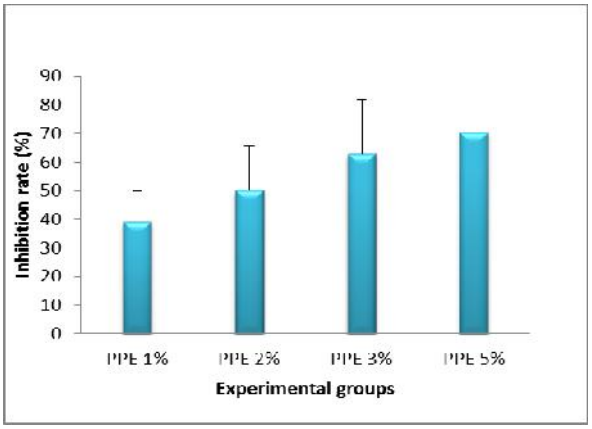

Figure 4.Means of mycelium inhibition rate in experimental groups exposed to PPE

Spore count evaluation. We also proceeded to the sporulation test of this fungus, according to our results we found a decrease in spore count compared to the control, for all extract concentrations tested (Table 2). This reduction corresponded to the mycelial growth decrease, due to their extract exposure, an inverse relationship was also found between the spores count and the extract concentration.

Table 2 . Spores count according to the different concentration of PPE

Groups	Spores count (.10^5)
Control	9
PPE1%	8.3
PPP2%	7.56
PPP3%	4.06
PPP5%	3.08

Many researchers highlight the PPE inhibitory activity against the mycelial growth germination of most plant fungal pathogens ,including *Botrytis cinerea*, *Penicillium digitatum*, *Penicillium expansum*, *Penicillium italicum*, *Alternaria alternata*, *Stemphylium botryosum*, *Colletotrichum acutatum sensu stricto*, *Fusarium oxysporum*, *Aspergillus parasiticus*, *Monilinia laxa* and *Monilinia fructigena* (Tayel et al., 2009; Oraki et al., 2011; Glazer et al., 2012; Rongai et al., 2015; Li Destri Nicosia et al., 2016; Rongai et al., 2017; Pangallo et al., 2017; Rongai et al., 2018).

Our results were coherent with those found by Al-Askar (2012), in which pomegranate peels significantly reduced the *Fusarium oxysporum* growth at different graduated concentrations. However, Glazer et al. (2012) confirmed the inhibitory effect of aqueous PPE on mycelial growth of *Alternaria alternata*, *Stemphylium botryosum* and *Fusarium spp*. However, the extract is ineffective

against *Penicillium expansum*, *Penicillium digitatum* and *Botrytis cinerea*. Similarly, Sudharsan et al. (2019) confirmed a similar results regarding the conidial germination and hyphal growth of the mycotoxigenic fungi *Aspergillus flavus* and *Fusarium proliferatum* .

The mechanisms by which the PPE bioactive components exert their activity have not been completely elucidated **(**Al-Zoreky, 2009; Oraki et al., 2011; Nuamsetti et al., 2012). The level of antifungal activity can considerably vary depending on extract type and pathogen species. For example, an ethanolic PPE can completely inhibit the germination of *Botrytis cinerea* conidia and *Colletotrichum acutatum*, while it was less effective against *Penicillium digitatum* and *Penicillium expansum*, with a reduction rate of 91.0% and 82.7%, respectively (Li Destri Nicosia et al., 2016; Pangallo et al., 2017).

Several studies correlated the PPE antifungal activity to their high concentration of polyphenols, particularly punicalagins and ellagic acids. Rongai et al. (2012) found that punicalagins are responsible for the inhibition of the *Fusarium oxysporum f. sp. lycopersici* mycelial growth, and highlighted that PPE are among the most effective plant extracts in preventing the *Fusarium oxysporum* germination.

According to some studies (Foss et al., 2014; Akhtar et al., 2015). The PPE polyphenolic compounds have the ability to combine with fungal cell membrane proteins, increasing cell permeability, which causes cell death . Furthermore, the PPE can decrease the pH gradient around the cell membrane and cause the cell death by increasing permeability (Rongai et al., 2018; Singh et al., 2019). Morphological changes in hyphae, including curling, twisting, and collapsing (microscopic observation) were detected in *Fusarium sambucinum* mycelium treated with methanol PPE (Elsherbiny et al., 2016). Cell empty cavities and disintegration of cytoplasmic organelles were also observed. In addition, an abnormal mycelial structure was detected in *Monilinia laxa and Monilinia fructigena* species following treatment with PPE (Elkhetabi et al., 2020).

CONCLUSION

This study showed the clear antagonistic effect of PPE at different concentrations towards *F. oxysporum f. sp. radicis- lycopersici*, indeed, *in-vitro* confrontation tests (on culture medium) between *F.oxysporum f.sp.radicis- lycopersici* and PPE, revealed an inhibition of the tested pathogen mycelial growth. This inhibition is dose-dependent.

Based on these results, it is of primary interest to use PPE as a biological control agent against *Fusarium* root and crown blight of tomato caused by *F.oxysporum f. sp.radicis- lycopersici*, especially since there are relatively few chemicals active against this pathogen. On the other hand, it is necessary to test PPE on different phytopathogenic agents, in order to identify the action spectrum to develop an alternative to the use of synthetic fungicides, with the objective of substituting them with a biological treatments based on plant extracts.

REFERENCES

Akhtar, S., Ismail, T., Fraternale, D., and Sestili, P. (2015). Pomegranate peel and peel extracts: Chemistry and food features. Food Chemistry, 174, 417- 425.

Al-Askar, A. A. A. (2012). *In- vitro* antifungal activity of three Saudi plant extracts against some phytopathogenic fungi. Journal of Plant Protection *Research 52*, 458-462.

Al-Zoreky, N., (2009). Antimicrobial activity of pomegranate (*Punica granatum L.*) fruit peels. International Journal of Food Microbiology 134, 244-248.

Benzohra, I.E., Bendahmane, B., Labdi, M. and Youcef Bnekada, M. (2011). *In vitro* biocontrol using the antagonist *Trichoderma harzianum* against the Algerian isolates of *Ascochyta rabiei (Pass.) Labr.*, the Agent of Ascochyta blight in chickpea (*Cicer arietinum L.*). International Journal of Microbiology Research 2, 124-128.

Castillo, F., Hernández, D., Gallegos, G., Rodríguez, R. and Aguilar, C.N. (2012). Antifungal properties of bioactive compounds from plants. In: Fungicides for plant and animal diseases; Dhanaseckaran D., Thajuddin N., Panneerselvam A. (eds). 82-106. In Tech, Rijeka.

Cerqueira, M.D., Barcellos, H., Machado, P., Aires, J. and Dummer, D. (2016). Antifungal activity of plant extracts with potential to control plant pathogens in pineapple. Asian Pacific Journal of Tropical Biomedicine, Vol. 6, 26-31.

De Corato, U., Violab, E., Arcierib, G., Valeriob, V. and Zimbardica, F. (2016). Use of composted agro-energy co-products and agricultural residues against soil-borne pathogens in horticultural soil-less systems. Scientia Horticulturae, 210, pp. 166-179.

Du, L., Li, J., Zhang, X., Wang, L., Zhang, W., Yang, M. and Hou, C. (2019). Pomegranate peel polyphenols inhibits inflammation in LPS-induced RAW264.7 macrophages via the suppression of TLR4/NFB pathway activation. Food & Nutrition Research, 63, 1-2.

Elkhetabi, A., Lahlali, R., Askarne, L., Ezrari, S., El Ghadaroui, L., Tahiri, A., Hrusti´c, J. and Amiri, S. (2020). Efficacy assessment of pomegranate peel aqueous extract for brown rot (*Monilinia spp.)* disease control. Physiological and Molecular Plant Pathology, 110 ,101482.

Elsherbiny, E.A., Amin, B.H. and Baka, Z.A. (2016). Efficiency of pomegranate (*Punica granatum L.*) peels extract as a high potential natural tool towards Fusarium dry rot on potato tubers. Postharvest Biology and Technology, 111, 256-263.

Fones, H.N., Bebber, D.P., Chaloner, T.M., Kay, W.T., Steinberg, G. and Gurr, S.J. (2020). Threats to global food security from emerging fungal and oomycete crop pathogens. Nature Food, 1, 332-342.

Foss, S.R., Nakamura, C.V., Ueda-Nakamura, T., Cortez, D.A., Endo, E.H.and Dias Filho, B.P. (2014). Antifungal activity of pomegranate peel extract and isolated compound punicalagin against dermatophytes. Annals of Clinical Microbiology and Antimicrobials, 13-32.

Gill, H.K. and Garg, H. (2014). Pesticides: Environmental impacts and management strategies. In Pesticides-Toxic Aspects; IntechOpen: Rijeka, Croatia.

Glazer, I., Masaphy, S., Marciano, P., Bar-Ilan, I., Holland, D., Kerem, Z. and Amir, R. (20129. Partial identification of antifungal compounds from *Punica granatum* peel extracts. Journal of Agricultural and Food Chemistry, 60, 4841-4848.

Jamil, F.F., Haq, I., Sarwar, N., Alam, S.S., Khan, J.A., Hanif, M., Khan, I.A., Sarwar, M. and Haq, M.A. (2002). Screening of ten advanced chickpea lines for blight and wilt resistance. The Nucleus, 39, 95-100.

Kanoun, K., Abbouni, B., Boudissa, S., Bouhafs, N. and Seddiki, M. (2016). Study of the activity of *Punica granatum Linn*. leaves extracts on *Candida albicans* and *Rhodotorula spp* . Phytothérapie, 14 ,5-16.

Kanoun, K., Abbouni, B., Bénine, M. L., Benmahdi, F.Z. and Marouf, B., (2014a). Étude de l'efficacité de l'extrait éthanolique d'écorces de *Punica granatum linn* sur deux souches phytopathogenes :*Ascocyhta rabiei* (pass.) labr. et *Fusarium oxysporum f.sp.radicis -lycopersic*i. European Scientific Journal, 10 (12), 301-315.

Kanoun, K., Abbouni, B., Gabbés, S., Dellani, S. and Zizi, N. (2014 b). *In-vitro* Antibacterial Activity of Algerian Pomegranate (*Punica granatum linn*) Peels on Some Antibiotic Resistant Gram-Negative and Positive Bacterial Strains. Middle-East Journal of Scientific Research, 21 (9), 1579-1589.

Kolai, N., Saiah, F. and Boudia A., (2012). Effet inhibiteur *in- vitro* de l'huile essentielle d'*Artimesia herba alba* sur deux souches de *Fusarium oxysporum f. sp.radicis-lycopersici*. Algerian Journal of Arid Environment, 2 ,71-76 .

Larsen, A.E., Gaines, S.D .and Deschênes, O. (2017). Agricultural pesticide use and adverse birth outcomes in the San Joaquin Valley of California. Nature Communications, 8 ,1-9.

Leroux, P. and Credet, A. (1978). Etude de l'activité des fongicide. INRA. Versailles France. 12 p.

Li Destri Nicosia, M.G., Pangallo, S., Raphael, G., Romeo, F.V., Strano, M.C., Rapisarda, P., Droby, S. and Schena, L (2016). Control of postharvest fungal rots on citrus fruit and sweet cherries using a pomegranate peel extract. Postharvest Biology and Technology, 114,54-61.

Li, Y., Ye, T., Yang, F., Hu, M., Liang, L., He, H., Li, Z., Zeng, A., Li,Y. and Yao, Y. (2016). *Punica granatum* (pomegranate) peel extract exerts potent antitumor and anti-metastasis activity in thyroid cancer. RSC Advances, 6 , 84523–84535.

Mahlo, S.M., Eloff, J.N. and Mc Gaw, L.J., (2010). Antifungal activity of leaf extracts from South African trees against plant pathogens. Crop Protection, 29, 1529-1533.

Mari, M., Bautista-Baños, S. and Sivakumar, D. (2016). Decay control in the postharvest system: Role of microbial and plant volatile organic compounds. Postharvest Biology and Technology, 122,70-81.

Maslouhy, A. (1989). Contribution à l'étude *in-vitro* et in situ des antagonistes de *Fusarium oxysporum f.sp.albedinis*, agent causal du bayoud. PhD thesis, Cadi Ayyad University, Faculty of Science, Marrakech, 98 p.

Nuamsetti, T., Dechayuenyong, P. and Tantipaibulvut, S. (2012). Antibacterial activity of pomegranate fruit peels and arils. ScienceAsia, 38, 319-322.

Oraki, H.H., Yagar, H. and Isbilir, S.S. (2012). Comparison of antioxidant activities of juice, peel, and seed of pomegranate (*Punica granatum L.*) and inter-relationships with total phenolic, tannin, anthocyanin, and flavonoid contents. Food Science and Biotechnology, 21, 373-387.

Oraki, H.H., Demirci, A.S. and Gümüş, T. (2011). Antibacterial and antifungal activity of pomegranate (*Punica granatum L. cv*.) peel. Electronic Journal of Environmental, Agricultural and Food Chemistry, 10, 1958-1969.

Palou, L., Ali, A., Fallik, E. and Romanazzi, G. (2016). Plant-and animal-derived compounds as alternatives to conventional fungicides for the control of postharvest diseases of fresh horticultural produce. Postharvest Biology and Technology, 122, 41-52.

Pande, S., Sharma, M., Gaur, P.M., Tripathi, S., Kaur, L., Basandrai, A., Khan, T., Gowda, C.L.L. and Siddique, K.H.M. (2011). Development of screening techniques and identification of new sources of resistance to Ascochyta blight disease of chickpea. Australasian Plant Pathology, 40, 149-156.