AgroLife Scientific Journal - Volume 9, Number 2, 2020
ISSN 2285-5718; ISSN CD-ROM 2285-5726; ISSN ONLINE 2286-0126; ISSN-L 2285-5718

THE ANTIFUNGAL EFFECT OF SOME NATURAL AND SYNTHETIC CHEMICAL COMPOUNDS ON THE BITTER ROT APPLE

Cristina MIHĂESCU[1], Loredana Beatrice NEAGU FRĂSIN[2]

[1]University of Pitesti, 1 Targu din Vale Street, Pitesti, Romania
[2]Valahia University of Targoviste, 13 Sinaia Aley, Targoviste, Romania

Corresponding authors email: cristescu_cri@yahoo.com

Abstract

Bitter fruit rot is an extremely damaging disease in all countries where apple cultivation is predominant. The pathogen attacks the fruits, especially those that are injured or stored in conditions unsuitable for preservation. The biological material (apple)was collected from the Research Institute for Fruit Growing Pitesti Maracineni. Using derivatives of dithiocarbamic acid, cinnamic acid, carbamate ester, nicotinic acid and copper hydroxide, they were evaluated in order to control the colony growth of mycelium, at the concentrations recommended in agriculture by the specialized literature. Although the minimum inhibitory concentration of the chemical compounds on the growth of the pathogen has been established, in order to avoid the excessive use of these compounds. In the present study, the optimal dose of use of some plant extracts was used for crop protection. Mancozeb had a special efficacy in all the studied variants, and among the used extracts. The Capsicum extract at the concentration of 15 µg/ml inhibited the growth of mycelium with a value close to that of copper hydroxide 0.2% and higher than the pyraclostrobin + boscalid combination.

***Key words**: bitter rot apple, in vitro, fungicide, plant extracts.*

INTRODUCTION

The apple and pear cropsare very widespread with a share of about half of fruit production in Europe. In Romania, apple and pear orchards occupy an important place in domestic production being estimated at approximately 350 000 tons in 2019.

The *Golden Deliciosus, Starkrimson, Ionathan*, and *Idared* varieties are well known in southern Europe, while in the northern part the Elstar and Conference varieties are very popular (CBS, 2016). In the absence of phytosanitary treatments, the susceptibility of the host to the attack of key pathogens is high. Thus, pathogens such as brown spot and apple and pear blights (*Venturia inaequalis*), powdery mildew (*Podosphaera leucotricha*), and open branch cancer (*Nectria galligena*) can cause economic losses in favorable years up to 70% (McHardy, 1996; Sutton et al., 2014). In the absence of phytosanitary treatments and improper storage conditions, other pathogens may appear in the stored fruits: gray rot (*Botrytis cinerea),* brown rot (*Monilinia fructigena*), green mold (*Penicillium expansum*), black rot (*Sphaeropsis malorum*), and soft fruit rot (*Rhizopus stolonifer*).

Romania has variable environmental conditions, which favors the growth and development of *Colletotrichum* spp. to infect different hosts. *Colletorichum gloeosporioides, Colletorichum coccodes, Colletorichum falcatum, Colletorichum truncatum*, *Colletorichum acutatum*, and *Colletorichum lindemuthianum* are some important species reported to be associated with anthracnose disease in Romania. *Colletorichum gloeosporioides* is an important pathogen in many crop plantsin Romania. The species is polyphagous with various symptoms such as brownish brown spots, immersed in the substrate, with concentric brown or grey concentric areas.

In Romania, the best results were obtained in the control of brown rot with Chorus 75 WDG, Signum, Switch and Bellis 38 WG. In modern agriculture, the concept of integrated control involves the corroboration of all physical, chemical and biological methods for combating diseases in agroecosystems (Dunea et al., 2015; Dincă and Dunea, 2017).

Although the chemicalization of agriculture predominates, the principles of organic farming that protect useful fauna in agroecosystems (Dunea et al., 2019), do not induce the phenomenon of resistance to virulent strains and minimize pesticide residues that can accumulate in the soil should not be omitted from the protection programs (Desneux et al., 2007; Roubos et al., 2014).

Irrational application of non-selective pesticides in orchards leads to the phenomenon of resistance even to secondary pathogens, which normally do not cause damage to agricultural crops (Damos et al., 2015).

MATERIALS AND METHODS

Colletotrichum gloeosporioides was isolated from the infected fruit of apple collected from Arges region. The pathogen was isolated from the fruit and grown on differential culture media such as potato dextrose agar (PDA) and Czapek Dox. In all experiments, incubation was at 25°C. In our studies, five repetitions were used for each variant for a correct statistical correlation.

In vitro evaluation of fungicides against Colletotrichum gloeosporioides

The pathogen dynamics was followed by the use of conventional systemic and contact fungicides: mancozeb 80% in concentration of 0.15%, 0.2%, 0.3%, 0.4%, dimetomorph 90 g/kg and mancozeb 600 g/kg in concentration of 0.1%, 0.2%, 0.25%, 0.3%, boscalid 26.7% and piraclostrobin 6.7% in concentration of 0.05%, 0.1%, 0.15%, 0.2% and 50% copper hydroxidein concentration of 0.2%, 0.4%, 0.5%, and 0.6%.

The dynamics of *Colletotrichum gloeosporioides* pathogen growth was calculated using the Vincent's formula, the percentage of hyphae inhibition in the experimental variants (Vincent, 1947). For this, discs with a diameter of 1 cm were cut from the control samples and placed in the center of the Petri dishes with the variants with the fungicides studied in different concentrations. The samples were incubated 14 days at 25°C. The percentage of inhibition was calculated after certain time intervals: three days, seven days, and ten days, respectively.

$$I = (C-T)/C \times 100$$

where:

I = Per cent inhibition;

C = Radial growth in control;

T = Radial growth in treatment (fungicide).

Development of the microwave assisted extraction procedure (MAE)

The plant material represented by gooseberry, pepper and fern was used to obtain the extract. For microwave assisted extraction (MAE) plant material was preliminarily processed by drying, grinding and sieving (Brajesh et al., 2014). The extract was obtained using a microwave equipment, model NEOS - GR (Microwave Extraction System from Milestone Inc.), following the following experimental parameters: power, time, and temperature.

Following the establishment of the experimental protocol, a single experimental variant was performed using 12.5 g of plant dissolved in 300 ml of solvent (water: alcohol), at a power of 312 W, for 10 minutes (Horbowicz, 2002). To highlight the antifungal efficacy of extracts, it was incorporated into the medium in different concentrations using three experimental variants: 5 $\mu g \cdot ml^{-1}$, 10$\mu g \cdot ml^{-1}$, and 15 $\mu g \cdot ml^{-1}$.

RESULTS AND DISCUSSIONS

The *Colletotrichum gloeosporioides* pathogen grown on PDA culture medium (potato glucose agar) forms whitish colonies with a radial development from the inoculation point, with regular margin. Mycelial hyphae are hyaline, superficial, septate, and branched. This culture medium is rich in dextrose, due to which the growth of mycelial hyphae was very fast; in four days, the diameter of the colony was on average 4.5/3.9 cm, and after ten days, the surface of the Petri dish was completely covered. In our *in vitro* observations, we also observed a color change of the colonies from palegrey with white concentric zones to uniform grey throughout the mass after about two weeks (Figure 1). Being a coelomycetes, the fungus form sconidiomata acervular, separate in concentric zones; the conidia from the culture measured 8-15 × 3- 4.5µm. More or less, Wang et al. (2012) observed the same trend. On the medium Czapek Dox, the fungus had a slow growth, the colonies having a white color, with irregular margins, the diameter being 1/1cm at 3 days and 3.5/4.8 cm at 10 days. The sporulation of the fungus on this medium is weak compared to the sporulation on the PDA; the conidia from the culture measured 6-11 × 3-5µm.

Figure 1. *Colletotrichum gloeosporioides* - control on PDA

Therefore, the mancozeb $(C_4H_6N_2S_4Mn)a\cdot(C_4H_4N_2S_4Zn)_y$ is a derivative of dithiocarbamic acid with well-known fungicidal properties. The exact mode of biological activity of these products is still little known. They are thought to be involved in cellular metabolism through their breakdown products, which have the ability to inactivate the thiol and sulfhydryl groups of amino acids. However mancozeb non systemic fungicide showed 100% inhibition at 0.15%, 0.2%, 0.3%, 0.4% concentrations (Figure 2).

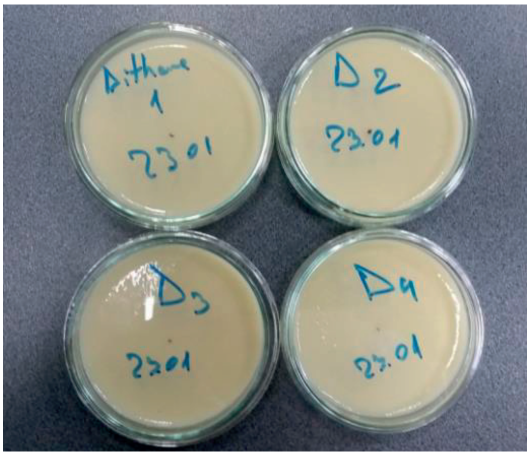

Figure 2. *C. gloeosporioides* - variant with mancozeb

The copper hydroxide CuH_2O_2 showed 100% inhibition at 0.4%, 0.5%, 0.6%. The bactericidal effect of cupric compounds is due to the action of copper ions, which combined with the thiol groups of organic molecules, is able to inactivate the enzymes and coenzymes involved in the cellular respiration. In addition to this direct action, copper salts, acting as abiotic elicitors, induce the synthesis of phytoalexins, which are substances endowed with the ability to inhibit a wide range of microorganisms (Figure 5).

Similar results were obtained by Solano and Arauz (1995) who applied mancozeb and captan against papaya anthracnose (*C. gloeosporioides*), and found that fungicides are very effective against the pathogen.

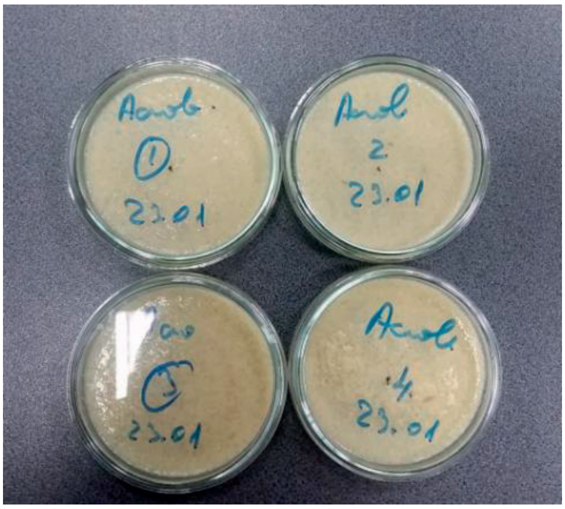

Figure 3. *C. gloeosporioides* - variant mancozeb + dimetomorph

Gawande et al. (2006) have reported that bordeaux mixture (1%) has a very good fungicidal effect on the pathogen, inhibiting mycelia growth by 90.7%.

The combination mancozeb + dimetomorph $(C_4H_6N_2S_4Mn)a\ (C_4H_4N_2S_4Zn)_y + C_{21}H_{22}ClNO_4$ showed toxic fungicidal effect at all concentrations (0.1%, 0.2%, 0.25% and 0.3%). Dimetomorph is a cinnamic acid derivative that acts systemically by inhibiting the formation of cell walls, having preventive, curative, and antisporulating effects. In combination with mancozeb, the fungicidal activity is exceptional (Figure 3).

Mancozeb is a fungicide with a multisite action, manifesting a contact action on the tissues of the host plant. During the pathogenesis process, the action is effective only in prevention. In curative control programs, mancozeb is associated with systemic synthetic products, achieving a double protection of crops and avoiding the phenomenon of resistance.

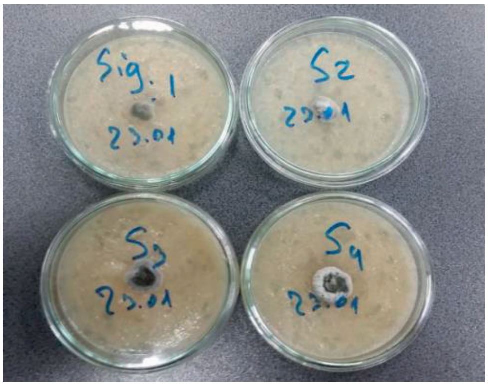

Figure 4. *C. gloeosporioides* - variant piraclostrobin + boscalid

The long-term strategy of using fungicides recommends the use of mixtures, as shown by us in laboratory observations. Along with dimethomorph, mancozeb has an excellent fungicidal action compared to even lower concentrations than recommended. Studies in this regard have shown that to avoid the emergence of resistant strains of *Plasmoparaviticola*, mancozeb is associated with folpet, aluminum fossil or azoxystrobins (Sierotzki et al., 2008).

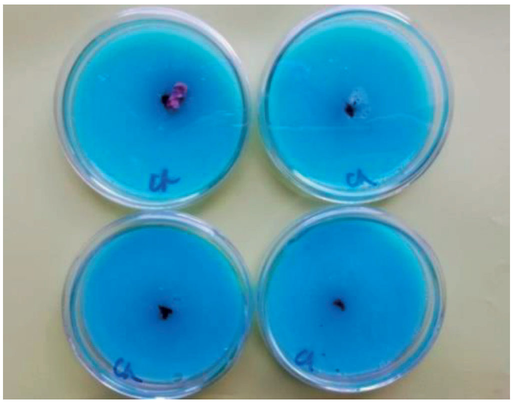

Figure 5. *C. gloeosporioides* - variant with copper

The efficacy of pyraclostrobin + boscalid $C_{19}H_{18}ClN_3O_4$ + $C_{18}H_{12}Cl_2N_2O$ increased with the increase in the concentration (Figure 4). At 0.1% and 0.15% and 0.2% pyraclostrobin + boscalid slightly inhibited the mycelial growth (mean 49.84% inhibition of mycelia growth), while at the concentration of an 0.05% improvement of the inhibition rate found around 32.76%.

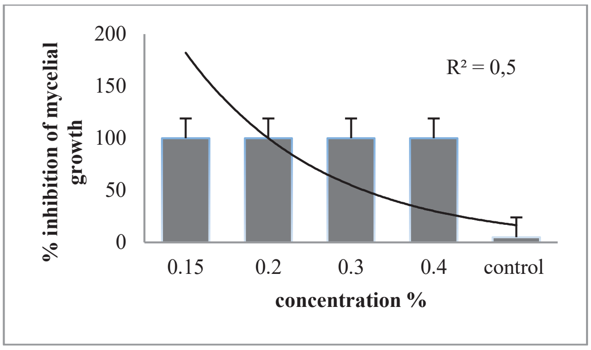

Figure 6. *In vitro* efficacy of mancozeb at different concentrations on mycelia growth of *C. gloeosporioides*

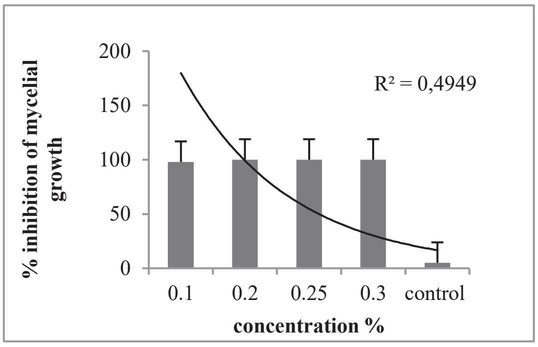

Figure 7. *In vitro* efficacy of mancozeb + dimetomorph at different concentrations on mycelia growth of *C. gloeosporioides*

The treatments with pyraclostrobin + boscalid have a progressive efficacy depending on the concentration used, which is also confirmed by the studies of Mari M. et al. (2009) on the species *Colletotrichum acutatum* in strawberry, where the percentage of mycelium inhibition was on average 60%.

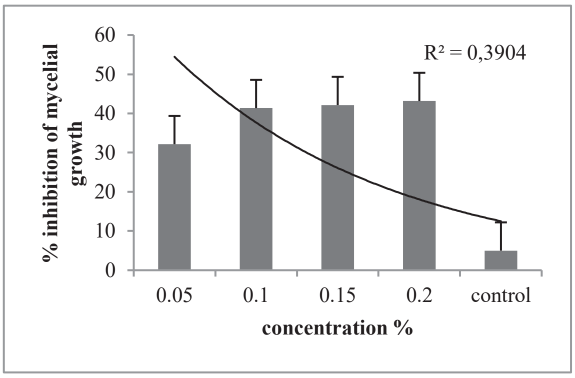

Figure 8. *In vitro* efficacy of piraclostrobin + boscalidat different concentrations on mycelia growth of *C. gloeosporioides*

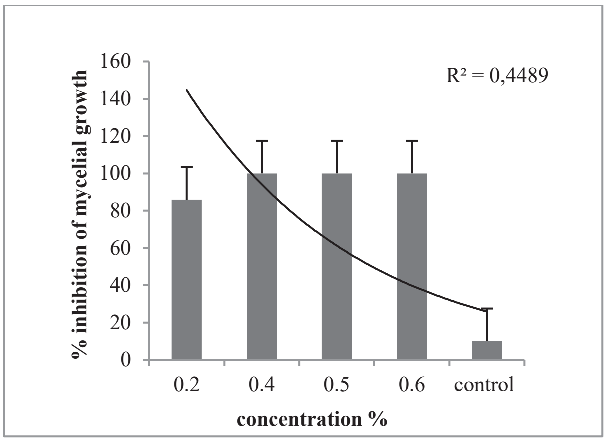

Figure 9. *In vitro* efficacy of copper hydroxide at different concentrations on mycelia growth of *C. gloeosporioides*

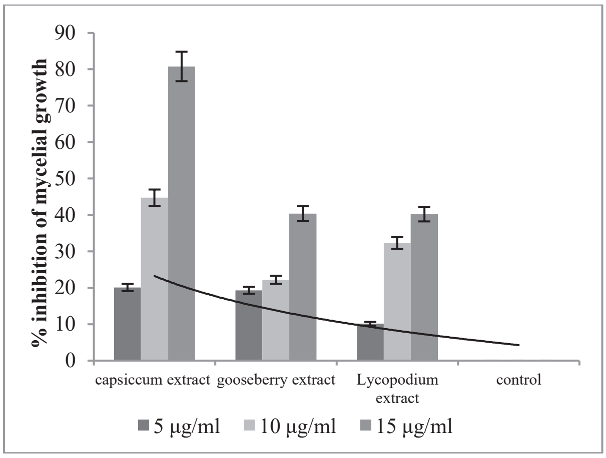

Figure 10. *In vitro* efficacy of plant extracts at different concentration of mycelia growth of *C. gloeosporioides*

Mancozeb had an inhibitory action on the fungal hyphae by 100% in all used concentrations (Figure 8). The percentage of 100% inhibition was also observed in the case of copper hydroxide variants 0.4-0.6% (Figure 9) and mancozeb + dimetomorph 0.2-0.3% (Figure 7).

The fungicidal or fungistatic effect on the pathogen *C. gloeosporioides* was also tested in the case of the ethyl extract of *Ribes*, *Capsicum* and *Lycopodium* in concentrations of 5 $µg·ml^{-1}$, 10 $µg·ml^{-1}$, and 15 $µg·ml^{-1}$. From the analysis of the graph, the *Capsicum* extract at the concentration of 15 µg/ml had a remarkable fungicidal effect on the growth of the pathogen, the inhibition rate being 80.78%. There are significant differences in the action of *Capsicum* extract 15 µg/ml on the inhibition of mycelial hyphae and currant and *Lycopodium* extracts, where in all variants the fungistatic effect is weak (Figure 10).

Similar results were obtained in establishing the fungicidal or fungistatic effect of *Cymbopogon citratus* essential oil on pathogens. At a low concentration of 0.25 mg/ml of essential oil, the mycelial hyphae were inhibited in a favorable percentage (Duarte, 2010).

The ecological alternative of using phytopreparations to combat bacterial and fungal diseases is increasingly agreed in agriculture. Spraying essential oils on leaf surfaces and treating fruits during storage with extracts from various plants have proven beneficial effects in combating a considerable number of pathogens (Aly et al., 2013; Sivakumar et al., 2014).

CONCLUSIONS

The *in vitro* efficacy of synthetic chemical compounds has shown that dimetomorph + mancozeb and mancozeb are highly effective against this pathogen, and it is recommended to be used in the treatment of fruits before storage. Biological control of pathogens is a modern alternative for the current agricultural practices. In this context, our studies focused on finding biological products with fungistatic or fungicidal effect on the pathogen under study. In this sense, only the *Capsicum* extract turned out to be efficient compared to other lower concentrations of *Capsicum* or the extracts of *Ribes* and *Lonicera* that were suitable for the control variant.

REFERENCES

Aly, A.A., Mohamed, H.I., Mansour M.T.M., Omar, M.R. (2013). Suppression of powdery mildew on flax by foliar application of essential oils. *J Phytopathol.,* 161(6), 376-381.

Brajesh, K., Kumari, S., Kumar, B., Cumbal, L., Rosero, G. (2014). Microwave-assisted extraction and solid-phase separation of quercetin from solid onion (*Allium cepa* L.), *Separation Science and Technology*, 49, 2502-2509.

Damos, P., Escudero, Colomar, L-A., Ioriatti, C. (2015). Integrated fruit production and pest management in Europe: The apple case study and how far we are from the original concept, *Insects*, 6, 626-657.

Desneux, N., Decourtye, A., Delpuech, J-M. (2007). The sublethal effects of pesticides on beneficial arthropods. *Annual Review of Entomology*, 52, 81-106.

Dincă, N., Dunea, D. (2017). The influence of *Rhizobium* inoculation and nitrogen/molybdenum fertilization on the growth characteristics of red clover. *AgroLife Scientific Journal*, 6(2), 83-88.

Duarte A.N., Schmidt, F.L., Duarte C.T., Figueira G.M., Delarmelina, C., Benato, E.A., Sartoratto, A. (2010). Control of *Colletotrichum gloeosporioides* in yellow passion fruit using *Cymbopogon citratus* essential oil. *Braz. J. Microbiol.*, 41(1).

Dunea, D., Dincă, N., Stanciu, A.M., Mihăescu, C. (2019). Determination of light absorption patterns within alfalfa varieties in Gherghita Plain. *AgroLife Scientific Journal*, 8(2), 40-47.

Dunea, D., Neagu Frăsin, L.B., Dincă, N. (2015). Ecophysiological responses of white clover - hybrid ryegrass mixture to foliar fertilisation. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 43(1), 173-178.

Gawande, A.D., Asalmol, M.N., Raut, S.P. (2006). Management of *Colletotrichum* spp. on *Piper longum*, *Linn. Annals Plant Prot. Sci,* 14**(**2), 411-414.

Horbowicz, M. (2002). Method of quercetin extraction from dry scales of onion. *Veget. Crops Res. Bull*., 57, 119-124.

Mari, M., Gengotti, S., Ceredi, G., Antoniacci, L., Montuschi, C. (2009). Control strategies for *Colletotrichum acutatum* on strawberries in north Italy ISHS, *Acta Horticulturae*, 842(842), 299-302.

McHardy, W.E. (1996). Apple scab: Biology, epidemiology and management. 545 p. APS Press, *The American Phytopathological Society, St Paul, Minnesota, USA.*

Roubos, C.R., Rodriguez-Saona, C., Holdcraft, R., Mason, K.S., Isaacs, R. (2014). Relative toxicity and residual activity of insecticides used in blueberry pest management: mortality of natural enemies. *Journal of Economic Entomology*, 107, 277-285.

Sierotzki, H., Kraus, N., Pepin, S., Fernandes, N., Gisi, U. (2008). Dynamics of QoI resistance in *Plasmopara viticola*. Pages 151-157 in: *Modern Fungicides and Antifungal Compounds IV.* H. W. Dehne, H.B. Deising, U. Gisi, K.H. Kuck, P.E. Russell, and H. Lyr, eds. 14th Int. Reinhardsbrunn Sympos. British Crop Protection Council, Farnham, Surrey.

Sivakumar, D., Bautista-Baños, S. (2014). A review on the use of essential oils for postharvest decay control and maintenance of fruit quality during storage. *Crop Protect*., 64, 27-37.

Solano, V., Arauz, L.F. (1995). Combating anthracnose in papaya fruit through fungicide application in Atlantic region of Costa Rica. *Agronomia Costarricense*, 19, 25-30.

Sutton, T.B., Aldwinckle, H.S., Agnello, A.M., Walgenbach, J.F. (2014). *Compendium of apple and pear diseases and pests.* APS Press.

Vincent, J.M. (1947). The esters of 4-hydroxybenzoic acid and related compounds. Part I. Methods for the study of their fungistatic properties. *J. Soc. Chem. Ind*., 6, 149-155.

Wang, C.X., Zhang, Z.F., Li, B.H., Wang, H.Y., Dong, X.L. (2012). First report of *Glomerella* leaf spot of apple caused by *Glomerella cingulata* in China. *Plant Dis*., 96, 912.

***CBS (2016). Statistics Netherlands. http://www.cbs.nl/en-GB/menu/home/default.htm.