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RESEARCH ARTICLE

Identification of biochemical composition and pesticides residue in ten rose cultivars leaves using FT-IR spectroscopy

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ABSTRACT

In order to optimize the application of nutritive solutions and the phytosanitary treatments with direct influence on the quality of cut roses from hydroponic culture (soilless culture), it is necessary to study the biochemical composition of the different organs of the plant (leaves). By FT-IR spectroscopy using the standard KBr pellet technique; it was possible to differentiate between 10 Rosa cultivars based on the chemical composition of the leaves and also to identify the pesticide residue within the leaf tissues and some of its effects. Thus, it was observed that the intensity of the bands corresponding to amide I (proteins) and aromatic compounds were weaker in leaf sheath and petiole compared with the leaf lamina. Aldehydes were present only in leaf sheath and petiole and not in the leaf lamina. As for the six pesticides tested, simple bond vibration C–H corresponding to aromatic compounds has been detected at 817 cm^{-1} and 805 cm^{-1} in the samples treated with penconazole based pesticide (Topas) and a triadimenol with folpet pesticide (Shavit). Also, application of penconazole increases the concentration of polisaccharides in the treated leaves, while a strong intensity band that could be attributed to the presence of halogenated compounds (chlorinated) C-Cl can be observed at 715 cm^{-1} for the samples treated with the triadimenol and folpet pesticide. The overall results of the present study could provide helpful information for the development of pesticides that have a shorter persistence and a lower biochemical influence on the plant tissues.

Keywords: Leaves tissue; Pesticide residues; Rose

INTRODUCTION

From about 20000 cultivars of roses today, the classes “Floribunda” and “Hybrid Tea roses” are the predominant gardens as well as greenhouse cut-flower production roses. From these two, the Hybrid Tea roses have larger flowers, and have been selected for their reliable recurring blooming, multi-petalled flowers (generally 25 to 35 petals), wide color range, long cutting stems and strong neck (Office of the Gene Technology AU, 2005). For commercial cultures roses can be grown in hydroponic system. This technology of cultivating roses for cut flowers became more popular world-wide since 1995. By optimizing the water and fertilization, hydroponic plants can produce more high quality flowers per square meter than soil-grown plants. However, in such a competitive environment as the cut flower market growers have to find a strategy to prevent the injury of the flower bud and top foliage from insects, mites and disease and at the same time avoid excessive or inadequate applications of pesticides that firstly tend to increase the pest resistance, and secondly it is known for a long time that most pesticides that are applied to the foliage have negative impact on the photosynthesis (Joshel and Melnicoe, 2004). Not at last it must be noted that there is a human health concern regarding the pesticide residue on the cut flowers (Morse et al., 1979, Toumi et al., 2016), and quick tests to assess the presence and quantity of pesticides in petals and leaves of cut roses have been developed (Cochran et al., 2011).

Today, hundreds of pesticides are used in agricultural practices in the world, and it is not unusual to find residues of these

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compounds in food products, flowers, fruits and vegetables (Vilca et al. 2018). Lentola et al. (2017) showed that many ornamental plants are also treated with systemic pesticides prior to purchase and there is little information as to whether these pesticides persist in plant tissues long enough to contaminate pollen during flowering after purchase. However, a recent report published by Greenpeace described the pesticides found in the leaves of 35 popular ornamental garden plants sourced from garden center in 10 European (but not UK) countries; pesticide residues were found in 97% of these flowering plants.

In order to optimize the application of phytosanitary treatments and nutritive solutions, it is necessary to study the persistence of natural and synthetic compounds in the organs of the plant. This could be realized by using modern and innovative technologies. Infrared (IR) spectroscopy is recognized as one of the best methods for identification of polar functional groups within the structure of organic compounds (Segneanu, 2012), especially in determination of the chemical group structure of unknown composition (Pillai and Nair, 2014).

At present time FT-IR method has been successfully utilized in the characterization of bacterial, fungal and plant species. In phytochemistry, FT-IR has been utilized to identify the concrete specific structure of certain plant secondary metabolites (Nazneen et al., 2012). Also, the FT-IR spectra revealed dramatic differences between plants, which indicated the variations in lipid metabolism, carbohydrate composition, and protein conformation from one genera to another. Even more, some studies successfully used this technique to identify the same species from different geographical regions, indicating that FT-IR represents a method for rapid and accurate molecular characterization and identification of plants based on the compositional and structural differences in their macromolecules (Gorgulu and Severcan, 2007). Previous FT-IR spectra study of dried leaves from various medicinal species, revealed that fourteen bands originating from different groups (N-H, O-H, C-H, C=O, C=C and C=N) could be considered as key bands. One can follow these key absorption bands characteristics and differentiate individual plant leaves or tissue. The manifestation of bands in the infrared region results primarily from the chlorophyll *a* and *b* molecules masking or suppressing the appearance of all other bands, which if they appear at all, they do with extremely low intensities (Konwar and Baruah 2011). As for the application of FI-TR in horticulture, FT-IR attenuated total reflectance technique can be used for *in situ* monitoring of plant physiological processes such as leaf senescence and aging or the plant responsiveness to fungal toxins (Ivanova and Singh, 2003). Also, portable FT-IR equipment to identify either illegal or counterfeit pesticides has been developed and is in use (Shannon and Rein, 2013) while detection of certain pesticide traces in horticultural products using FT-IR has been already proven an effective method (Guangdong et al., 2015).

In the present study the biochemical characterization of *Rosa* sp. leaves was realized using FT-IR spectroscopy. The aim of this research was to provide proof of concept for the use of FT-IR technique as tool for comparative biochemical characterization of *Rosa* cultivars and for highlighting the potential of this technique to identify pesticide traces in cut flowers. Two objectives were defined:

1) Identification of spectral regions corresponding to the main constituents from *Rosa* sp. leaves and screening for potential differences between cultivars;
2) Assessment of the potential of FT-IR technique to identify particularities associated with pesticide traces in leaves.

MATERIALS AND METHODS

The experiments regarding biochemical characterization of *Rosa* sp. leaves using FT-IR spectroscopy were conducted in the Raman and IR Spectrometry laboratory of the Institute for Life Sciences from University of Agricultural Sciences and Veterinary Medicine (UASMV) Cluj-Napoca during 2018-2020.

The biologic material was represented by 10 *Rosa* cultivars from the group "Thea hybrida" cultivated in hydroponic system (on coconut peat) by the company IRIS Cluj-Napoca.

The ten cultivars selected for this study were: 'Avalanche', 'Revue', 'Marina', 'Demetra', 'Good Times', 'Testarossa', 'Bordeaux', 'Samba', 'Chic' and 'Merci' which come from Kordes Rosen company, Germany.

Technical material

The samples were comprised of fragments of mature leaves (leaf blade, leaf sheath and petiole) belonging to the 10 cultivars, harvested from plants that underwent treatments at intervals of 7-12 days, with the following pesticides (Table 1): Mirage 0.2%, Novadim 0.2%, Score

Table 1: Chemical products used for pest control in the experiment regarding the pesticide residue in rose cultivars leaves

Pesticide (commercial name)	Action	Active ingredient (according to label)
Mirage	Systemic fungicide	Prochloraz
Novadim	Insecticide	Dimethoate
Score	Systemic fungicide	Difenoconazole
Shavit	Complex fungicide	Triadimenol and folpet
Topas	Systemtic fungicide	Penconazole
Vertimec	Insecticide and acaricide	Abamectin and cyclohexanol

0.05%, Shavit 0,2%, Topas 0.25%, Vertimec 0.2%. The control samples were comprised of leaves collected from plants that did not underwent any phytosanitary treatments. The purpose of the analysis was to differentiate between the cultivars studied based on the chemical composition of the leaves (proteins, aromatic compounds, esters, aldehydes, carbohydrates), as well as identifying the residue of the pesticides within the leaf tissues by FT-IR (Fourier transform infrared spectroscopy).

The samples were analyzed using FT-IR, a quick technique to differentiate between cultivars based on leaf fragments (Gorgulu et al., 2007). The equipment used in this experiment was a spectrometer FT/IR-4100 (Jasco Analytical Instruments – Easton, USA), having the spectral region between 4000-350 cm^{-1} and a resolution of 4 cm^{-1}.

To determine the chemical composition, 10 mature leaves from 5 plants belonging to the ten *Rosa* cultivars were harvested. The fresh harvested leaves were dehydrated in oven at 120 °C for 4 h, and the dehydrated plant material was grinded to powder, using mortar and pestle (Fig. 1). For each cultivar 50 leaves were dried.

The 3 mg powder was mixed with 200 mg potassium bromide (KBr) then compressed into spectral pellets (with Specac - IR accessory for producing pills) according to literature (http://www.niu.edu/, http://web.mst.edu/; Konwar and Baruah, 2011) (Fig. 2).

The pellets were scanned using Spectra Manager software. For each sample there were made 264 scans in 3 replicates. It the Fig. 2, the average scans can be observed. The primary data was subsequently analyzed with ORIGIN 8.5 Pro software.

In order to facilitate the spectra analysis of KBr pellets of pesticide treated leaves and identify the molecular fingerprint of the different residual pesticides within the leaf, besides the spectra of control samples, the spectra data obtained was compared with IR spectra from National Institute of Standards and Technology Chemistry WebBook for the active ingredients of the pesticides used in this study. The IR spectra from literature used belongs to: cyclohexanol and lactone (NIST Chemistry WebBook) for Vertimec since avermectins (UC IPM, http://ipm.ucanr.edu) are a family of macrocyclic lactones (Lasota and Dybas, 1990), penconazole the main active ingredient of Topas belongs to the group of 1,2,4-triazoles (Čadková et al., 2013) and spectra of this group was used (NIST Chemistry WebBook), triazole IR spectra corresponding to triadimenol and folpet spectra was compared with spectra of leaf samples treated with Shavit (NIST Chemistry WebBook), the dimethoate IR spectra (NIST Chemistry WebBook) for Novadim and imidazole (NIST Chemistry WebBook) for the prochloraz based product Mirage since prochloraz is an imidazole fungicide (Vinggaard et al., 2006), triazole IR spectra (NIST Chemistry WebBook) was compared with spectra of leaves treated with difenoconazole based product Score, since difenoconazole is essentially a triazole fungicide (Dong et al., 2013).

RESULTS AND DISCUSSIONS

Determination of biochemical composition of leaf lamina using FT-IR

The data from Fig. 3, shows a wide variability of vital chemical compounds (proteins, lipids and carbohydrates) identified in the analyzed samples. The results are in accordance with other authors (Gorgulu et al., 2007; Järvinen et al., 2011; Ivanova and Singh, 2003).

To facilitate the characterization of spectral absorption bands, three regions have been delimited as follows: first region 1800-1500 cm^{-1}, second region 1500-850 cm^{-1} and respectively third region 500-850 cm^{-1}.

The first region, between 1800-1500 cm^{-1}, is characterized by the presence of a band at about ≈1735 cm^{-1} for 'Samba', 'Merci', 'Demetra' and at 1731 cm^{-1} for the cultivars 'Marina' and 'Chic'. These bands have been attributed

Fig 1. Grinding rose leaves and placing the resulting powder in Eppendorf tubes to determine the chemical composition and the pesticide residues

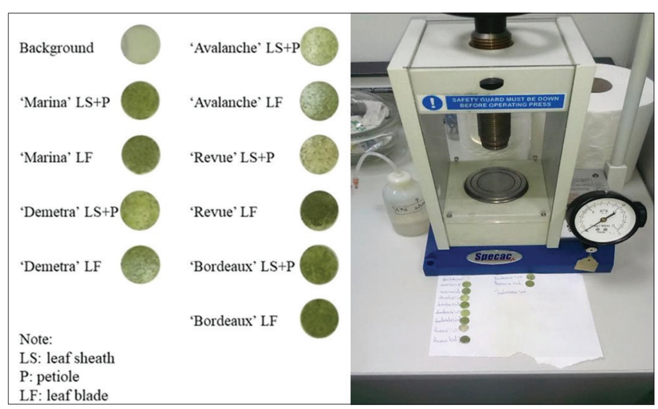

Fig 2. Preparing the KBr spectral pellets using Specac - IR accessory

to the stretching vibrations of carbon to oxygen double bonds (C=O), that indicate the presence of esters, which has been demonstrated by Järvinen et al. (2011), as well as by Chen et al. (2013).

The band from ≈1650 cm^{-1}, in most analyzed samples is described as a C=O stretching vibration, that corresponds to the presence of amide I (proteins and pectines), observed also by Gorgulu et al. (2007) in *Citrus* leaves. Similar research results have been obtained by Ivanova and Singh (2003).

In the same spectral region, the aromatic compounds present in the leaves can be observed at about 1560 cm^{-1} (Ivanova and Singh, 2003) for the cultivar 'Chic', while for other analyzed samples, similar vibrations occurred at 1559 cm^{-1} ('Marina'), 1558 cm^{-1} ('Good Time' and 'Testarossa').

Stretching C–N vibration in combination with N–H bending from the region 1554 cm^{-1} ('Revue', 'Merci' and 'Avalanche') are attributed to the presence of amide II (proteins), which was proved by Gorgolu el al. (2007) as well as by Skotti et al. (2014).

In the second spectral region (1500-850 cm^{-1}), a prominent band appears around wave number ≈1454 cm^{-1}, attributed to the C–OH bending (Chen et al., 2013). This band has the highest intensity for the samples 'Revue' and 'Chic'.

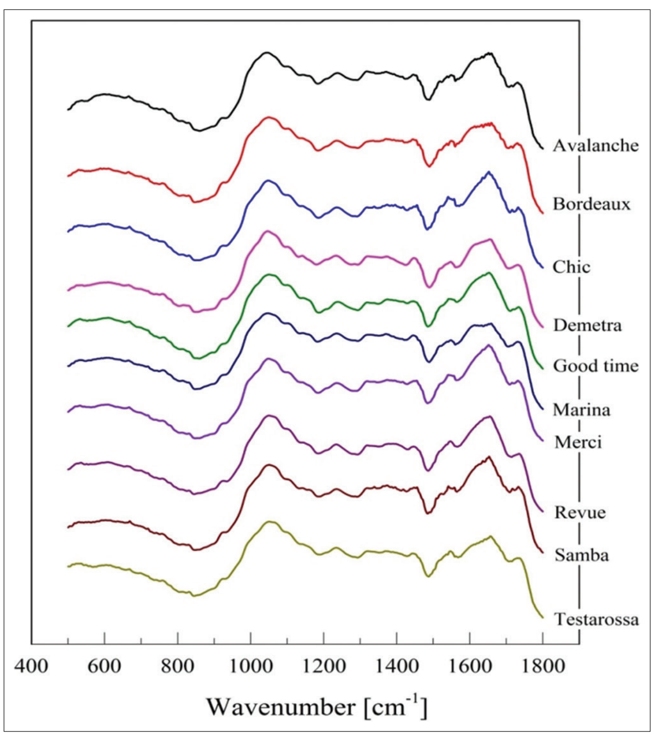

Fig 3. FT-IR absorption bands in the spectral region 1800-500 cm^{-1} (resolution 4 cm^{-1}) for leaf blade (lamina) samples belonging to 10 cultivars of *Rosa*

Weak vibrations appear around 1315 cm^{-1} for majority of the samples analyzed. This band could indicate the presence of amide III (proteins) (Skotti et al., 2014).

In spectral region 1200-900 cm^{-1} carbohydrates can be distinguished (Skotti et al., 2014) by C–O, C–OH and OH bond stretching. The ≈920 cm^{-1} band indicates probably the presence of starch, as some authors (Sankaran et al., 2010) indentified in *Gladiolus* leaves.

The band from 1037 cm^{-1} is described as having weak intensity and is attributed to the OH and C–OH bond stretching from the polysaccharides molecules (Gorgulu et al., 2007) for the cultivars 'Testarossa' and 'Good Time'.

The third region comprises the spectral bands from 850 to 500 cm^{-1}. More intense vibrations can be observed at 835 cm^{-1}, which indicates the presence of C–H and C–C bond bending of aromatic compounds (Järvinen et al., 2011; Chen et al, 2013).

Previous studies show the existence of different band intensities in the case of different species within the same genus (Gorgulu et al., 2007; Konwar and Baruah, 2011; Ivanova and Singh, 2003). However, these previous studies do not refer to different spectral bands in case of various cultivars within a given species.

Determination of biochemical composition of leaves (leaf sheath and petiole) using FT-IR

In order to describe the molecular vibrations of the ten *Rosa* cultivars studied under the influence of some pesticides, it was settled for analysis the spectral region between 1800-500 cm^{-1}. The distribution of the spectral bands for the 10 samples of rose leaf sheath and petiole, can be observed in the Fig. 4. To facilitate the characterization of spectral absorption bands, three regions have been delimited as follows: first region 1800-1500 cm^{-1}, second region 1500-850 cm^{-1} and respectively third region 500-850 cm^{-1}.

The detailed analysis of the molecular vibrations shows that the first spectral area of interest from the region 1800-1500 cm^{-1}, is the band present at 1734 cm^{-1}. This vibration seems better defined in the case of cultivars 'Testarossa' and 'Merci'. This band indicates probably the presence of esters as Järvinen et al. (2011) and Chen et al. (2013) have demonstrated.

Also the presence of amide I (proteins) can be noted, as it was observed by Gorgulu et al., (2007) as well as by Ivanova and Singh (2003) in various ornamental plants, caused by C=O stretching vibrations and in the case of cultivars 'Revue', 'Demetra' and 'Bordeaux' appears around 1657 cm^{-1}. The intensity of the bands is weak, fact that can be correlated with a smaller quantity of proteins in leaf sheath and petiole compared with the leaf lamina (Figs. 3 and 4).

The spectral band from 1614 cm^{-1} is more visible in 'Avalanche' and 'Good Time', while in 'Revue' appears at 1618 cm^{-1}. This band is described as a stretching of the double bond C=O, indicating the presence of aldehydes (Konwar and Baruah, 2011). These compounds have not been identified in the leaf lamina.

At about 1560 cm^{-1} appears the domain of aromatic compounds (Ivanov and Singh, 2003; Chen et al. 2013). It can be observed that the bands from 1559 cm^{-1}, 1560 cm^{-1} and 1562 cm^{-1} have a weaker intensity in leaf sheath and petiole, while in leaf lamina the molecular vibration intensity of the same compounds was much stronger. The absorption band from 1511 cm^{-1} it is present for 'Avalanche' and almost with same way (1514 cm^{-1}) for 'Bordeaux', 1515 cm^{-1} for 'Revue' and 1517 cm^{-1} for 'Marina'.

The second spectral region is located between 1500-850 cm^{-1}. The cultivars 'Avalanche', 'Bordeaux', 'Demetra' and 'Revue' present C–OH bond bending vibrations at about ≈1453 cm^{-1}.The C–N and N–H vibration bands from the region 1316 cm^{-1} are specific for amide III (proteins) (Skotti et al., 2014).

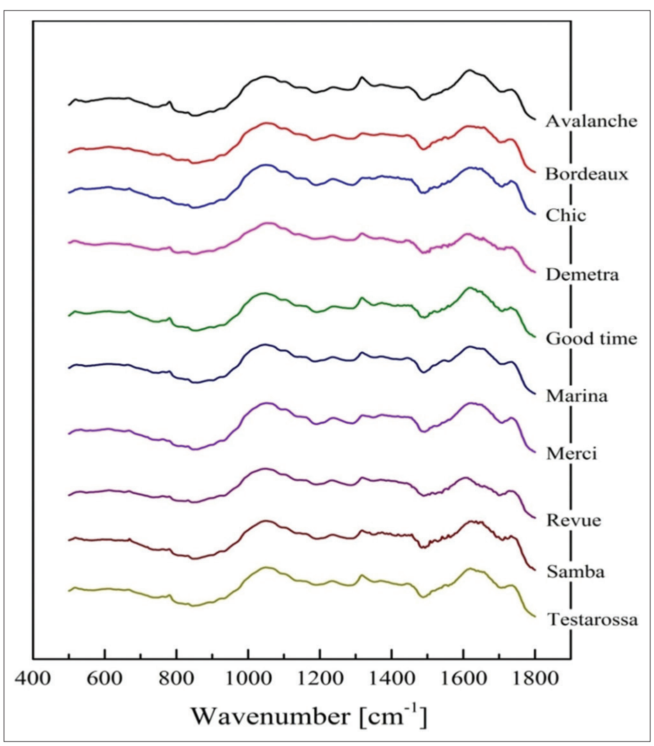

Fig 4. Assignment of FT-IR spectral bands in the region 1800-500 cm^{-1} (resolution 4 cm^{-1}) for the samples (leaf sheath and petiole) belonging to 10 *Rosa* cultivars

In the spectral area 1200 cm^{-1},cultivars 'Samba' (1240 cm^{-1}), 'Merci' (1236 cm^{-1}) and 'Chic' (1238 cm^{-1}), present vibration bands attributed to the presence of carbohydrates (Skotti et al., 2014; Sankaran et al., 2010). Weaker vibrations can be noted for the cultivars 'Avalanche' (1245 cm^{-1}), 'Bordeaux' (1233 cm^{-1}) and 'Marina' (1231 cm^{-1}). The band from 1042 cm^{-1} can be described as a low intensity band, and is present in cultivars 'Samba' and 'Merci', and it is attributed as well to the presence of carbohydrates (Skotti et al., 2014) in leaf sheath and petiole samples.

Spectral region between 850-500 cm^{-1} is the third region considered for analysis. The stronger vibrations can be observed around the bands 835 cm^{-1} for 'Marina', followed by 833 cm^{-1} for 'Samba', 'Merci', 'Bordeaux', and respectively 831 cm^{-1} for 'Chic'. These C–H, C–C bending vibrations, signal the presence of aromatic compounds (Järvinen et al., 2011; Chen et al., 2013).

Identification of pesticide residues through molecular vibrations method (FT-IR)

The phytosanitary treatments (for pests and diseases) can influence the biochemical composition of plants, and respectively of the roses leaves, through their content in bioactive ingredients. The ratio of chemical and aromatic compounds can be settled using molecular vibrations, and the obtained results could be used to intervene in favor of pesticides with lower persistence.

In the Fig. 5, it can be observed the vibration bands of the leaf samples treated with six pesticides (Mirage, Novadim, Score, Shavit, Topas, Vertimec) (Table 1). The analyzed leaves underwent treatments at 7-12 days intervals.

To facilitate the characterization of spectral absorption bands, three regions have been delimited as follows: first

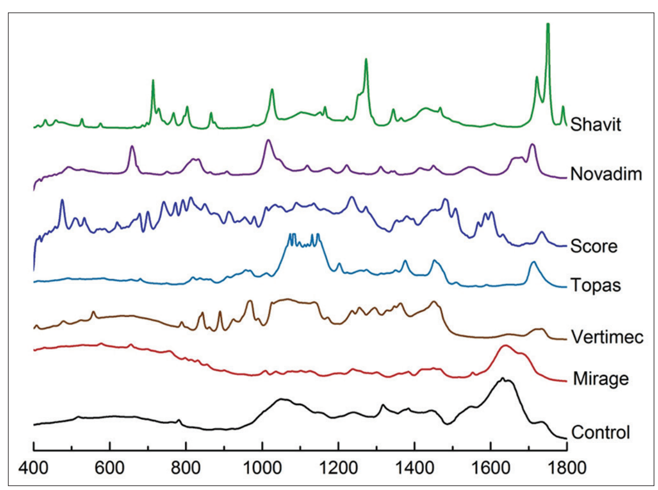

Fig 5. Molecular vibrations for leaf samples treated with six different pesticides, from the region 1800-500 cm^{-1} (resolution 4 cm^{-1})

region 1800-1500 cm^{-1}, second region 1500-850 cm^{-1} and respectively third region 500-850 cm^{-1}.

The first spectral region for the samples treated with Shavit, the strong molecular vibrations appear at 1790 cm^{-1} and 1750 cm^{-1}. Spectral bands between 1538-1818 cm^{-1} can be attributed to the presence of water (Sankaran et al., 2010).

In the case of the product Topas, strong vibrations appear at 1717 cm^{-1}. The band from 1710 cm^{-1} is weak and appears for the sample treated with Novadim.

It can be noted that in the case of the products Shavit and Topas the water content is higher in the leaves or the absorption of water could be higher. The results obtained by Lentola et al., (2017) also indicate that neonicotinoids are widely used for treatment of ornamental plants and their residues could contaminate gardens and parks.

In the second analyzed region, the spectral band from ≈1451 cm^{-1}, can be attributed to to the C–H bending vibrations of the aromatic compounds (Chen et al., 2013). The most intense spectral band is present at 1450 cm^{-1}, for the sample treated with the product Topas.

Weak vibrations can be observed for the samples treated with Vertimec at the band 1450 cm^{-1}. In the case of the rest of the samples this vibration is absent.

Strong molecular vibrations appear at 1375 cm^{-1} in the samples treated with the product Topas. This vibration could be due to CH_3 bending vibrations (Chen et al., 2013).

Another strong vibration can be noted at the band ≈1273 cm^{-1} for the samples treated with Shavit. This band is atributed to bond stretching vibrations of C–O, an indicator for esters that was demonstrated by Chen et al. (2013).

Also, in this spectral region multiple vibrations occur in the carbohydrates area (1200-900 cm^{-1}). The strongest vibrations appear for the samples treated with Topas (1070-1147 cm^{-1}). That application of this product increases the concentration of polysaccharides in the treated leaves. The peaks 1026 cm^{-1} (Shavit) and 1015 cm^{-1} (Novadim) are attributed to the presence of carbohydrates, although their intensity is weaker than for the product Topas.

The third spectral region is characterized by the presence of stronger signals in area 805-835 cm^{-1}, attributed to the C–H simple bonds from the aromatic compounds, confirmed by Herredia-Guerrero et al. (2014). Strong vibrations have been detected at 817 cm^{-1} and 805 cm^{-1} in the samples treated with the products Topas and Shavit. Another strong intensity band can be observed at 715 cm^{-1} for the samples treated with Shavit; this band could be attributed to the presence of halogenated compounds (chlorinated) C-Cl, described by Deepashree et al. 2013.

In the context of an increasing trend for the use of plant protection products as previsioned for the next decades (Taylor et al., 2021) it becomes essential to develop precise methods to investigate their presence and identify their pathways in the environment. It has been recognized that the fate of pesticides is insufficiently understood (Meftaul et al., 2020; Taylor et al., 2021). Pesticide traces in ornamentals can have cascading consequence for the environment and human health, it was demonstrated that can affect directly or indirectly the environment through horticulture runoff (Ali et al., 2021). Also, pesticide residues can negative impact on the natural biodiversity leading to the degradation of the ecosystems, including decline of pollinators (Lentola et al., 2017). Pesticides can accumulate across food chains and in excessive amounts can become a serious health threat (Debnath and Khan 2017; Ali et al., 2021).

It can be stated that according to the World Floriculture Map (Van Rijswick, 2015) data register until 2013 highlights that Netherlands (52%) is the largest cut flower export nation, followed by Columbia (15%), and Ecuador (9%) (https://www.trademap.org/Index.aspx) but more than 70% of Ethiopia's floriculture products, mostly roses, go to the Dutch market and from there these flowers are re-exported to other EU countries (Mengistie et al., 2017).

Worldwide about 200,000 hectares are cultivated with cut flowers (Toumi et al., 2016). Among these, roses, carnations and chrysanthemums representing the most important cut flower crops (Toumi et al., 2016; Darras, 2021). European Union is a leading market for cut flower sales with an increasing trend in last years. Life cycle assessment approach indicates that entire value-chain of cut roses either produced in countries from Africa or Europe needs to shift towards sustainable means of production, giving their forecast environmental footprint (Darras, 2020).

Even if flower industry was affected by the ongoing COVID-19 pandemic (https://www.floraldaily.com/article/9319845/the-ethiopian-flower-industry-has-shown-remarkable-growth-over-the-past-years/), the global Cut Flowers market was valued at 30100 million US$ in 2018 and will reach 46300 million US$ by the end of 2025 according to Cut Flowers Industry Outlook Analysis 2021 (https://www.360marketupdates.com/global-cut-flowers-market-13849629).

The flower crops are subject to treatments with pesticides that contain chemicals ranging in toxicity and hazard class, but ornamentals received less scrutiny for the amounts of pesticides residues in them (Shentema et al., 2020). The use of pesticides to maintain plant quality represents an important strategy adopted by the growers of ornamental plants to remain competitive (Wei and Khachatryan, 2021), and this can be a hindrance at implementing responsible stewardship regarding pesticide use.

Literature on the topic of exposure of workers in the sector across the chain of flower production to market, has received some attention in past decade (Morse et al., 1979; Carlile, 2006; Lu and Cosca, 2011; Enserink et al. 2013; Stanley and Raine, 2016; Mengistie et al., 2017; Brouwer et al., 2010; Toumi et al., 2016a; Toumi et al., 2018; Shentema et al., 2020; Nassar and Ribeiro, 2020; Aprea et al., 2021). Different studies conclude problems causing the workers' feet to swell due to standing for many hours in the greenhouse, kidney problems headaches, coughing, skin rushes, respiratory problems, blood vein problems, pneumonia, bronchitis, sinus, vomiting and others (Mekonnen and Agonafir 2002; Negatu et al. 2016; Mengistie et al., 2016; Mengistie et al., 2017) and poorer neurobehavioral development, reproductive disorders, congenital malformations and genotoxicity have been reported for residents of flower production areas and workers throughout the flower production cycle (Pereira et al., 2021).

A study conducted in rose flower farms in Ethiopia identified abnormal serum cholinesterase levels in over 10% of the workers across farms that might be linked to exposure to pesticides at different stages of production including handling harvested flowers (Shentema et al., 2020). A study from Netherlands showed that greenhouse workers experience a mean dermal exposure rate to pesticide compounds through cutting and sorting/budding carnations between 10.1-7.3 mg/hour (Brouwer et al., 2010). A study conducted on cut roses bouquets sold in some shops from seven cities from Belgium, detected 97 active pesticide substances in roses stem samples, with an average of 14 actives substances/bouquet, while most of these reached high levels of about 10-50 mg/kg (Toumi et al., 2016b). A further study demonstrated that florists are exposed to pesticides through handling cut flowers while pesticide identified on gloves used in the experiment during work time were also detected in their urine (Toumi et al., 2018; Toumi et al., 2019). Another study from South Korea, revealed that the amount of organophosphorus and pyrethroid pesticide exposure was highest in flower workers followed by wholesale florists and retail florists (Song et al., 2014).

The body of research dealing with adverse effects on human health due to exposure to pesticides applied to flower production as presented above and sources herein, outlines the need of sustained efforts directed towards reduction of pesticide presence in ornamentals, besides the required strategies to mitigate both the human exposure and the environmental impact.

Also, according research studies chemicals released from flower farms in Ethiopia for example, can negatively affect the quality of water and aquatic, water and soil quality on non-target organisms like soil organisms, aquatic animals, human beings, and the increase of pesticide resistance of targeted pests are reported (Sisay, 2007; Tamiru, 2007; Sahle and Potting, 2013; Mengistie et al., 2017).

This paper provides a proof of concept for the use of FT-IR method to identify pesticides traces in rose's leaves. Given the fact that this method is minimally-invasive, it could be optimized for fast screening technique of cut flowers. The pesticides used in this study have systemic action, and by absorbing in the plant these are considered as highly efficient. Because cut flowers are not products destined for consumption, can be understood why systemic pesticides might be seen as efficient choice of use by many growers during the entire cultivation cycle. However, increased awareness among workers and growers might contribute to rational use of pesticides in flower production as well.

CONCLUSIONS

As many research result shown frequently arise question if the pesticides residue on cut flowers could have an impact on the health of florists or consumers and the answer that pesticide residues applied on flowers can generate detrimental health effects is reported by a large number of researches. For example, Lu (2005) based on some surveys observed that workers who re-entered a recently sprayed area were 20 times more likely to get ill than those who did not. Abell et al. (2000) found that male fecundity could be decreased after exposure to pesticides in the manual handling of ornamental flowers in greenhouses. Therefore, the subject concerning the pesticide residues on health and environment is with global interest.

Although, The Federal Institute for Risk Assessment from Germany (Bundesinstitut für Risikobewertung - BfR) has recently evaluated the situation about residues on cut flowers. Based on the current state of knowledge and considering scientific publications, the BfR concludes that, there is no health risk for florists and consumers due to residues of plant protection products on ornamental plants (https://www.bfr.bund.de/cm/349/assessment-of-health-risks-from-pesticide-residues-on-cut-flowers.pdf.).

The emerging health and environmental concerns point to the need of sustainable and co-friendly approaches in regards with cut flower cultivation. Because pesticides are integral part of the successful cultivation of this category of plants and the quality and quantity of the flowers depends on it, the pesticides will continue to be used in foreseeable future. By monitoring and regulating appropriately the pesticide traces in ornamentals and retail cut-flowers, their amounts could be ensured to remain within lower threshold levels.

Roses are one of the most important cut flower crops with increasing demand. This research provides a FT-IR characterization of leaf lamina, sheath and petiole of ten *Rosa* sp. cultivars for cut flowers, grown in hydroponic system and provides proof of concept for the use of this technique to identify traces of pesticides in leaves.

After the KBr pellets analysis with method FT-IT, of leaf lamina, sheath and petiole belonging to 10 *Rosa* cultivars treated with six pesticides frequently used in the soilless culture of cut roses, the following conclusions can be drawn: The main bioactive molecules (proteins and carbohydrates) are present in all samples analyzed. The quantitative differences among the analyzed samples are due to different vibration intensities of the bands studied. In leaf lamina, besides proteins and carbohydrates, the esters and aromatic compounds have been identified. In the leaf sheath and petiole proteins and carbohydrates are present, although in lower quantities compared to leaf lamina. It can be noted the presence of aldehydes in the leaf sheath and petiole, compounds that have not been identified in the leaf lamina. The aromatic compounds present bands of weak intensity in leaf sheath and petiole. In the leaf lamina samples, these compounds present stronger molecular vibrations.

The pesticides used in this study were able to influence the biochemical composition of the leaf samples in different ways. Under the influence of the products Shavit and Topas, the water content of the leaf increases. The presence of aromatic compounds is more pronounced in the samples treated with Vertimec. The product Shavit can influence the presence of esters and can determine the presence of halogenated compounds (chlorinated), in the treated samples. In the case of the application of the product Topas the polysaccharide content in leaves increases.

Based on the results and the current state of knowledge, there is the possibility that pesticide to be source and risks of contamination, but more studies are required to demonstrate this. It is important to know exactly the level of pesticide residues in main organs of plants, because some ornamentals are frequently used in culinary art, perfume and pharmaceutical industry, and small quantities of these substances can influence human health.

AUTHORS CONTRIBUTIONS

All authors have contributed to the work, and they have agreed to submit the manuscript. Prof. Maria Cantor, PhD, is the coordinator of the research team and the responsible for the structure of this scientific work. Associate Professor, Erzsebet Buta, PhD, is responsible for writing the scientific paper and monitoring the research work. Ioana Conțiu, PhD, Ioana Crișan, PhD, Assistant Professor, Timea Buru, PhD, are the heart of this work by conducting experiments and Professor Răzvan Ștefan, PhD, contribute by interpreting the results of current research work.

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