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Pesticide residues surveillance and anomalies monitoring of 'Maltaise demi sanguine' (*Citrus sinensis* L.) oranges in packinghouses

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Abstract - For the monitoring of pesticide residues during the two agricultural seasons 2012-2013 and 2013-2014, one hundred and forty-two 'Maltaise demi sanguine' orange samples were collected from packinghouses. Sample analysis carried out by extraction procedure based on QuECHERS method followed by UHPLC-MS/MS chromatographic analysis showed that on both campaigns malathion, dimethoate and methidation were the only active ingredients that exceeded the Maximum Residue Levels (MRL) set by the European Union. The frequency of the MRL's excess varied through the seasons and methidathion decreased from 85.71% to 80%, malathion declined from 54.55% to 36.36%. However, dimethoate increased from 62.5% to 75%. The upper value of concentration of each molecule varied too showing a decrease in the case of methidathion from 540 to 180 ppb and an increase in malathion from 38.64 to 93.57 ppb.
Besides these tests, monitoring the export of 'Maltaise' oranges had shown the importance of maintenance of citrus orchards and the importance of the operation of collection to minimize the physical damages that are on the top of other post-harvest deteriorations and which decline fruit during the last operation of selection in the packing chain of oranges and represent sources of infection by fungi.

Keywords: Oranges, pesticides, UHPLC-MS/MS, sorting gap, agricultural practices.

1. Introduction

Pesticides are widely used in agriculture to control pests, plant diseases and weeds. They are a necessary tool to provide high crop yields and high quality of food products. However, pesticides are developed through very strict regulation processes to function with reasonable certainty and minimal impact on human health (Lavigene 2013).
Despite the regulations, serious concerns about health risks raised from successive exposure to these chemicals, or from their residues inhaled through air or ingested through food and water (Calvet and al. 2005). Yet, considerable amounts of harmful pesticide residues often remain in the harvested fruits, becoming a permanent danger to the consumer and creating health hazards ranging from short-term impacts such as headaches and nausea to chronic impacts like cancer, reproductive harm, and endocrine disruption (Camard and Magdelaine 2001).
Actually, the presence of pesticide residues in food receives more worldwide attention and the register and set of the Maximum Residue Limits (MRLs) is part of control authorities' responsibilities . These limits are established either by FAO and European Commission Union (EU) in order to protect the environment and particularly consumer health by minimizing pesticide concentration in fruits and vegetables proposed to consumer (EC 2005, FAO; UNEP; WHO 1991).
To control the respect of the code of Good Phytosanitary Practices, pesticides residue monitoring programs are the only tool to control the quantity of pesticides on food and to enforce tolerances (OMS

1988). In the case of Tunisia, a little information about the residues of pesticides in fruits and vegetables is available.
In this context, the aim of this present study is to monitor pesticide residues in oranges sampled from Tunisian packinghouses during two agricultural seasons 2012-2013 and 2013-2014 and to check compliance with existing regulations especially for European Community. Moreover, a surveillance of anomalies on the fruit attempts to determine the causes of sorting gaps and propose solutions to maintain the quality of oranges in export.

2. Materials and Methods

2.1. Samples and Sampling

The monitoring was conducted during two agricultural seasons (2012-2013) and (2013-2014) from January to March and from February to March respectively. The sampling concerned the 'Maltaise demi sanguine' oranges collected from 11 and 13 packing houses respectively located on the main production region of citrus Cap Bon.
The citrus sampling led to a total of 107 fruit samples from the first agricultural campaign and 39 fruit samples from the second one. They were performed in accordance with the guidelines of FAO (Miller 1995); which means that, the sample was taken at various places distributed throughout the lot. Orange samples weighted with a minimum of 1 kg and collected in bags. All samples were labeled and stocked at 4°C until the extraction was done.

2.2. Materials and Reagents

Acetonitrile Pestinorm for residue analysis was purchased from BDH Prolabo. Standards of selected active ingredients: abamectin, acetamipride, bifenazate, deltamethrine, diafenthuron, flufenoxuron, imidaclopride, lufenuron, propargite, malathion, spinosade, spirodiclofene, thiamethoxam, methidathion, spiromesifene, fenpyroximate, dimethoathe, chlorpyriphos-ethyl, fenoxycarbe, thiophanate-methyl and carbendazim were obtained from the stocks of the laboratory of Analysis of Pesticides in the Ministry of Agriculture. The individual stock solutions were prepared by dissolving 100 mg of each compound in 100 mL of acetonitrile, except for the carbendazim, which was prepared in 10 M HCl instead of acetonitrile. Then they were stored in glass stopper bottles at 4°C. Standard working solutions at various concentrations were prepared daily by appropriate dilution of aliquots of stock solution in acetonitrile.

2.3. Extraction procedure

The extraction procedure is based on QuEChERS method published by Anastassiades and al. (2003). This method follows AFNOR standard NF EN 15662 (2009) and is validated to use in the Laboratory of Analysis of Pesticides in the Ministry of Agriculture in Tunisia.
Samples collected were coarsely chopped and homogenized for 3 min at high speed using a laboratory food chopper to generate uniform sample representative of the product. 10g of homogenized orange sample was weighted in a 50 mL flacon tube and 10 mL of acetonitrile Pestinorm was added to orange sample. Then, 4 g magnesium sulfate ($MgSO_4$), anhydrous; 1 g sodium chloride (NaCl); 1 g trisodium citrate dehydrate ($C_6H_5Na_3O_7.2H_2O$) and 0.5g disodium hydrogencitrate sesquihydrate ($C_6H_8Na_2O_8$) were added to the mixture for extraction. High speed vortex mixer was applied for mixing for one minute. Then, a centrifugation was realized for five minutes at 3500 *g*. The supernatant obtained was conserved in the refrigerator during 24 hours. The step of cleaning consisted in the removal of the supernatant in a 15 mL flacon tube containing 900mg of $MgSO_4$ and 150mg of Primary and Secondary Amino adsorbent (PSA). Once mixed, the extract is spin-dried at 3500 *g* during five minutes to separate the solid material. The supernatant is adjusted and stabilized with a 5% formic acid in acetonitrile solution. Finally, the stabilized extract is transferred to an autosampler vial in the refrigerator until chromatographic analysis (Anastassiades and al. 2003).

2.4. Chromatographic Analysis

Analysis of final extracts were carried out on a Shimadzu UHPLC-MS-MS system equipped with a binary solvent pumps, an autosampler, and a mass-selective detector with Electro-Spray Ionization (ESI) (Anastassiades and al. 2003) (Table 1).

Table 1: Conditions of the chromatographic analysis.

ChromatographicLiquid (LC)	
Column	Pinnacle DB AQ C18 (L=100mm*ϕ=2,1mm*1,9µm)
Mobile phase flow rate	0,2 mL/min
Temperatureoven/ column	40°C
Mobile phase	20% Me-OH 80% H_2O
Injection Volume	10µL
Mass-Spectrometry (MS-MS)	
Nebulizer Gas flow-rate	3L/min
Drying gas flow-rate	15L/min
Vaporizer temperature	400°C
Nebulizer Gas	Argon à 230KPa

2.5. Monitoring of 'Maltaise demi-sanguine' anomalies in packinghouses

This monitoring aimed the determination of the causes of the sorting gap fruit by counting the damages observed on oranges in four packing houses. For this analysis, six boxes from two different agricultures were selected randomly on each visit. Then, the fruit were analyzed one by one (Fourtassi 1998).
The packing houses were selected from a list supplied from the Ministry of Power Industry and Small and Medium Enterprises and the choices made were based on the quality and size of the packinghouses' exports. The visits were extended during the two campaigns from February until late March.

3. Results and discussion

3.1. Residue pesticides analysis

Samples analysis of oranges recovered from the packinghouses showed, during the first season 2012-2013, that 29.52 % of the samples were contaminated (Figure 1). In this context, 67.74 % of these samples had exceeded the Maximum Residue Levels (MRL) while 32.26 % showed no overtaking.

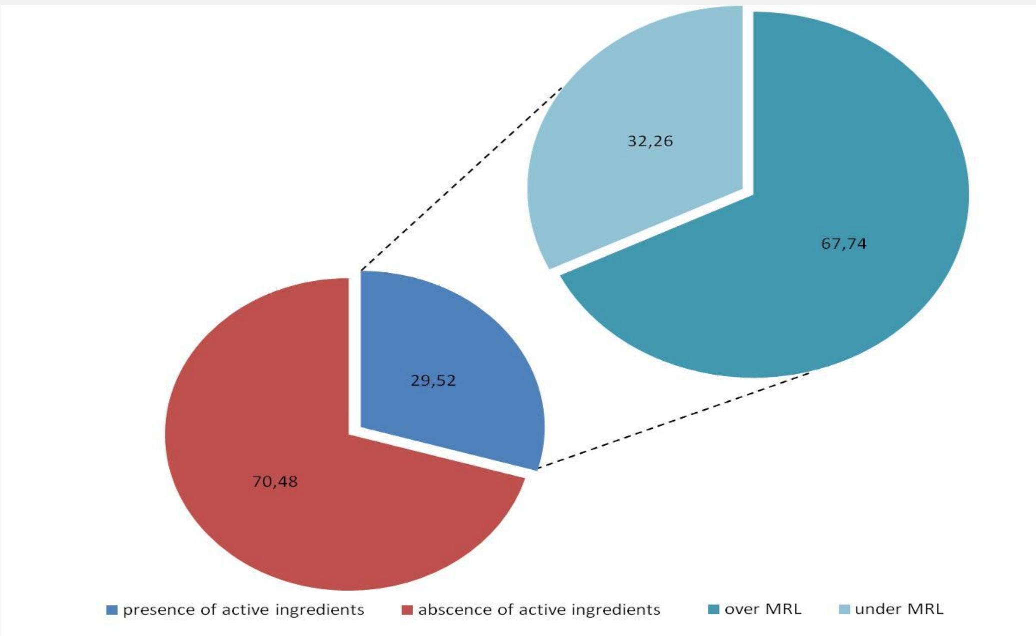

Figure 1: Packing houses residue analysis during the season 2012-2013.

In this case, the four detected active ingredients were malathion, methidathion, dimethoate and chlorpyrifos- ethyl present in 20.95, 6.66, 7.62 and 2.86 % of the analyzed samples (Figure 2) and their concentrations varied between 4.52 and 38,642 ppb for malathion, 6.252 and 540,640 ppb for methidathion, 10.97 and 111.421 ppb for dimethoate and 20.22 and 50,973 ppb for chlorpyrifos- ethyl. Compared with MRL set by the European Union (20 ppb), the three insecticides malathion, methidathion and dimethoate exceeded the MRL with a frequency of 11.43, 5.71 and 4.76 %.

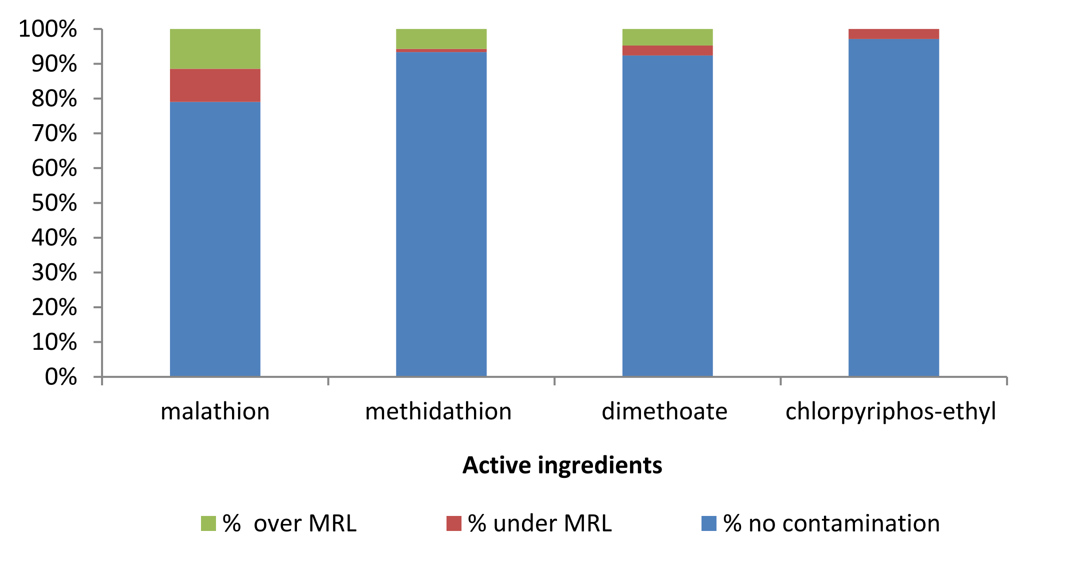

Figure 2: Frequencyof non-conformity of the active ingredients during the season 2012-2013.

During the second agricultural season 2013-2014, the analysis of the samples collected from the packing houses revealed an increase on contaminated samples (62.16 %) and a decrease on the active ingredients free ones (37.84 %) (Figure 3). However, the percentage of frequencies of MRL's excess in samples decreased from 67.74 % to 43.48 %.

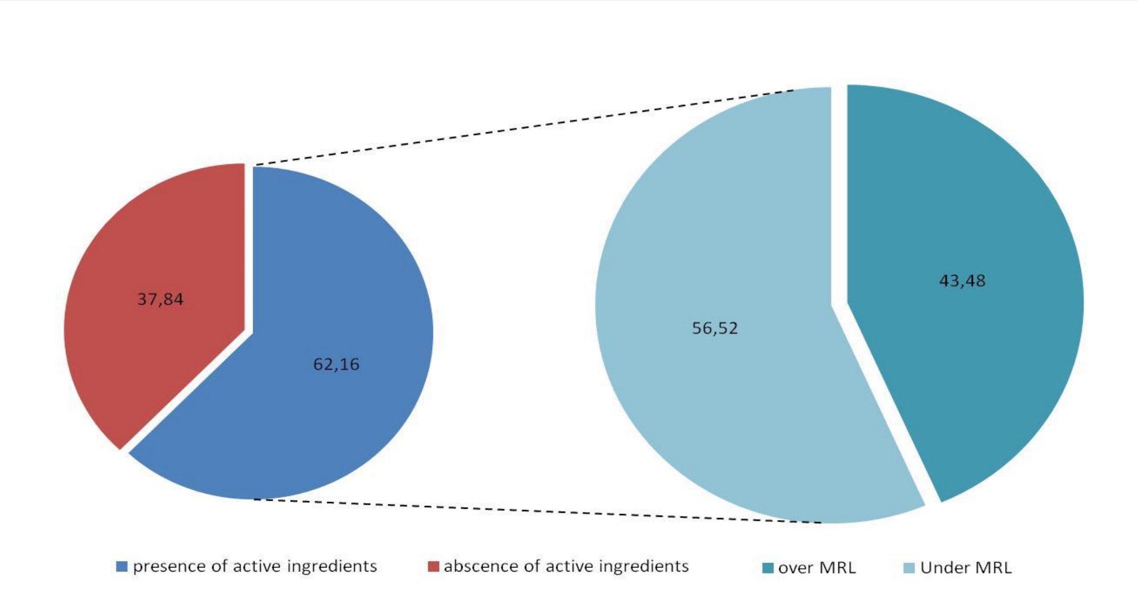

Figure 3: Packing houses residue analysis during the season 2013-2014.

Compared with the MRL set by the European Union, the results of samples analysis showed that, as the previous year , only malathion, methidathion and dimethoate exceeded the limit (Figure 4) even though, more active ingredients were present in the samples than in the first campaign: acetamiprid (15.78 ppb), malathion (10.26-93.57 ppb), spirodiclofen (10.62-18.77 ppb), methidathion (13.10-180.32 ppb), dimethoate (21.5-55.57 ppb), chlorpyrifos- ethyl (16.15-38.96 ppb), fenoxycarb (11.76 ppb) and thiophante -methyl (23.12 ppb).

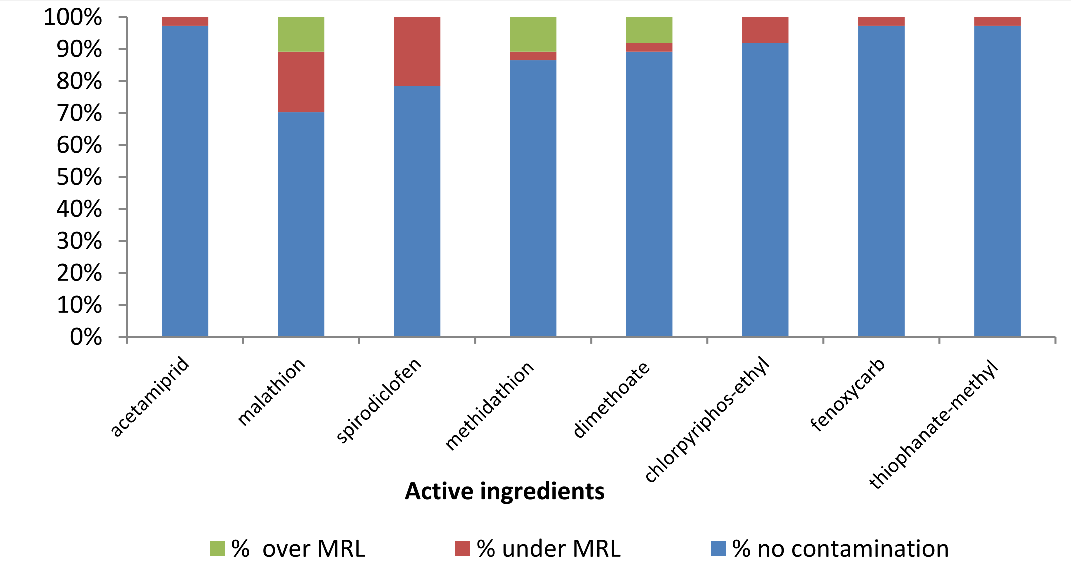

Figure 4: Frequency of non-conformity of the active ingredients during the season 2013-2014.

From the results obtained during the two campaigns (Figure 5), we notice that the frequency of MRL's excess of methidathion decreased from 85.71% to 80%. Similarly, the exceeding frequency of malathion declined from 54.55% to 36.36%. However, the frequency of oranges contaminated with dimethoate increased from 62.5% to 75%.

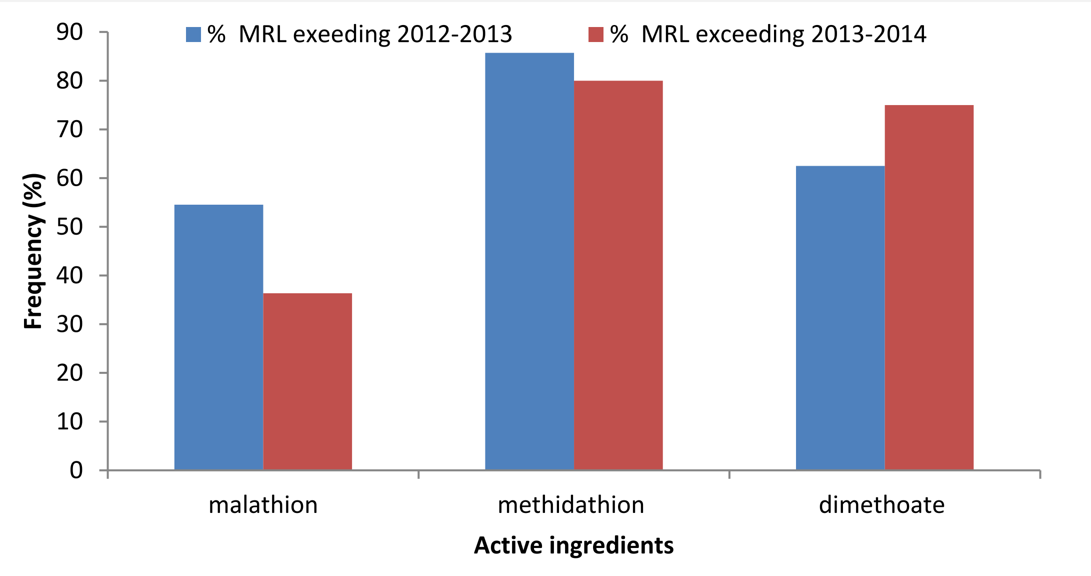

Figure 5: Frequency of MRL overtaking during both campaigns.

In the same context, the upper value of methidathion concentration considerably decreased from 540.64 to 180.32 ppb during the second campaign. This decrease could be due to the prohibition of the use of pesticides containing methidathion after the non acceptance of oranges contaminated with this active ingredient during the first campaign. However, the concentration of malathion increased from 38.64 to 93.57 ppb, despite the decrease of its frequency on the samples.
The excessive and unreasonable use of insecticides with the active ingredients malathion, dimethoate and methidathion, in order to protect oranges from the Mediterranean fruit fly, would be the basis of MRL's over pass. However, these aberrations caused the repression of oranges from European countries since some substances such as malathion are banned in Europe (El khaddam and al. 2013).Yet, on an

attempt to maintain the level of export of our oranges, the number of commercial preparations with malathion decreased from 11 to 3 preparations between 2008 and 2012 (Anonymous 2012). However, the prohibition of the use of methidathion after the first season has resulted in an increase on the use of malathion in the second one.

3.2. Monitoring of 'Maltaise demi-sanguine' anomalies in packing houses

Monitoring the campaign allowed the counting of anomalies observed on fruits. Their causes are diverse and vary according to the climatic conditions of the production area, the treatments made and the cultivation practices.

Among the problems found on fruits, some do not downgrade fruits in the last sorting phase of the packaging line while others decommission oranges but their presence does not affect the adjacent fruits and others can generate great damages.

These anomalies are mainly due to: physical and phytosanitary causes.

3.2.1. Physical Causes

The manipulation of fruits during the picking is generally the source of these anomalies. It conditions a high percentage of sorting gaps.

-- **Injuries**: they are caused by work tools, including the pliers used for picking, broken boxes or overfilled ones, badly cut nails gatherers, the shock of fruits during loading and transport; these injuries are the main causes of the proliferation of blue and green molds.

--**The stalk:** the presence of the stalk is a quality feature; its absence is an anomaly and may facilitate the proliferation of fungi such as *Penicillium italicum*. However, when the stalk is long, it can be a source of injuries to other oranges.

-- **Mottling:** the wind can cause frictions between fruits from fruit set causing the suberisation of the surface of the skin; the sand-laden winds can cause similar damage.

--**Oleocelosis:** it is due to physical damage on turgid fruits, oil-bearing glands release the essential oils they contain on the fruit surface which causes the necrosis and the destruction of surrounding skin cells. The affected portion sags slightly and turns brown. The oleocelosis is likely to occur when the fruit is harvested by cold, wet weather, or in newly irrigated land (Cuquerella, 1997).

-- **The small size:** it is due to the heavy load of trees leading to a competition between fruit, it occurs, especially, in cases of water shortage and a non- optimized technical management of the orchard.

3.2.2. Phytosanitary Causes

The lack of sustained observations in the orchard and of adequate phytosanitary treatments may cause the occurrence of pests or diseases that cause considerable damages to the trees and fruits.

-- **Pests:** mealybugs, mites and medfly form the group of the most dangerous enemies of citrus.

-- **Diseases:** those caused by fungi are the most common such as blue mold (*P. italicum*) and green mold (*P.digitatum*). These post- harvest diseases can cause considerable losses in packing houses and in cold storage since they attack the wounded fruits, hence the need to look after the harvesting operations. Moreover, sooty mold (*Capnodium citri*), which develops on the sweet secretions of aphids and scale insects strongly adheres to the fruit which makes its removal very difficult when washing.

These problems thus defined and after monitoring the various damages, the following can be retained from the physical (Figure 6) and phytosanitary (Figure 7) problems from both campaigns:

- Oleocelosis was the most downgrading factor of oranges. Then, hail caused countless damages on the fruits.
- Fallen fruit and the absence of the stalk which have been almost equal, during both seasons, were highly correlated according to the equation: $\mathbf{y = 1{,}058x - 1{,}408;\ R^2 = 0{,}968}$
- The frequency of small fruit size increased during the second season when the other problems decreased or remained almost stable.
- The frequency of long stalk was positively correlated to injured fruit according to the equation: $\mathbf{y = 0{,}778x - 1{,}934;\ R^2 = 0{,}412}$

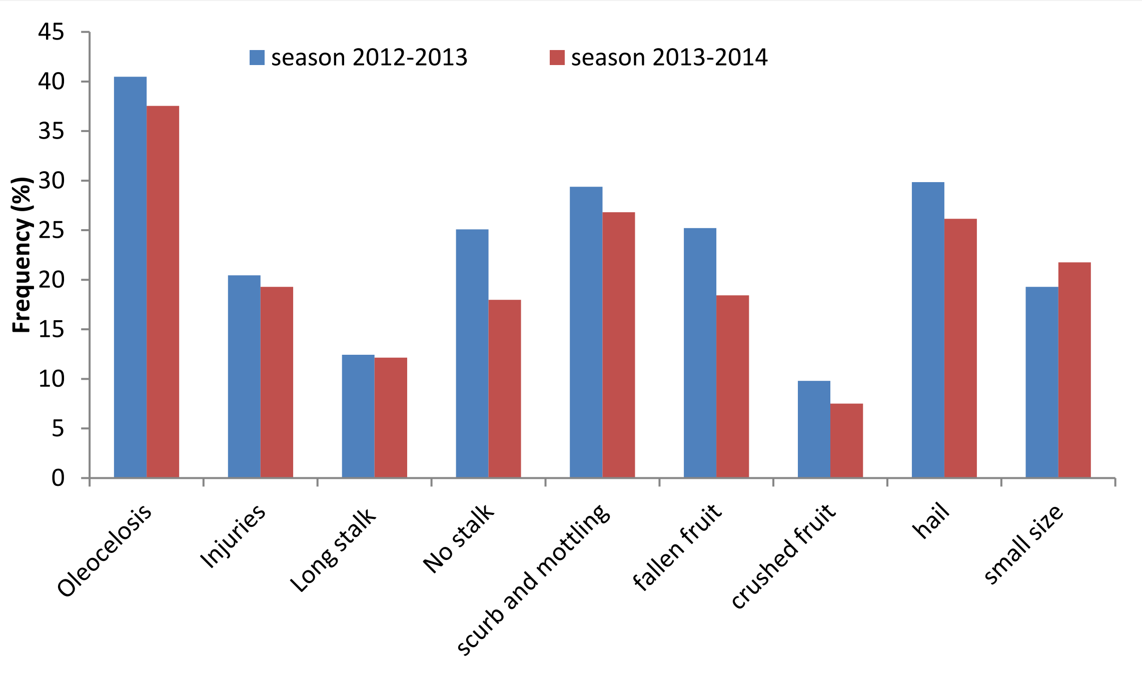

Figure 6: Physical damages on 'Maltaise demi sanguin' oranges.

- The fruit medfly was almost absent in the samples of both campaigns. This might be due to the excessive use of insecticides since the only cases of MRL exceed were with malathion, methidathion and dimethoate used to fight this insect.
- The presence of red mite was rare compared to that of the yellow tea-mite.
- In the case of decay which was mainly due to both *Penicillium* species, the percentage of attacks did not exceed 2.4% on both seasons. Whereas, this value is susceptible to be duplicated during the transit operations and/ or cold storage when injured fruits are left.

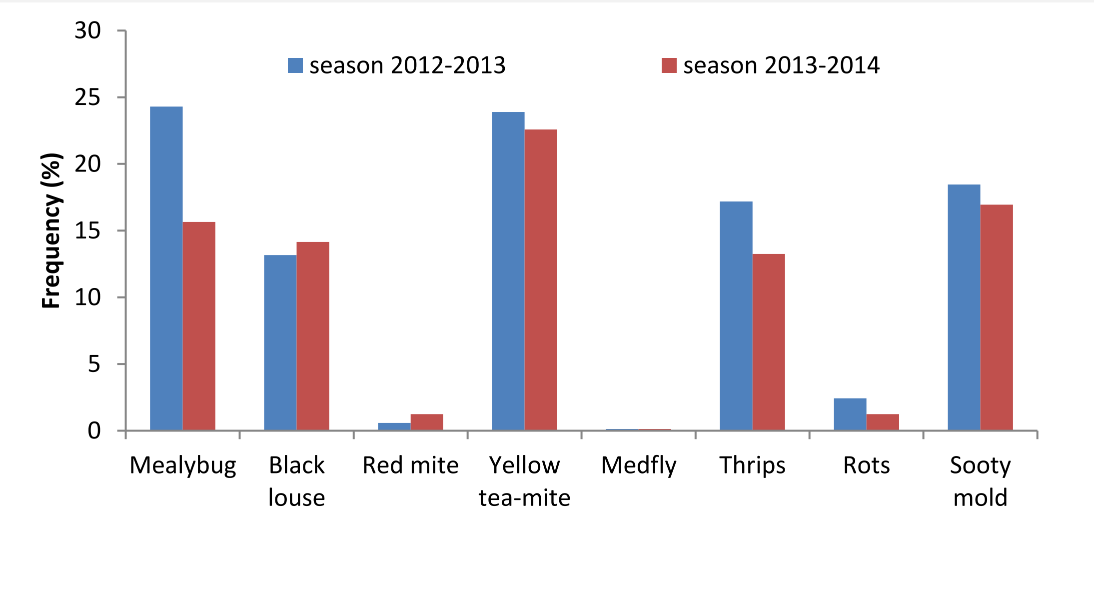

Figure 7: Phytosanitary damages on 'Maltaise demi sanguin' oranges.

Based on the results presented above, it is crucial to think of better supervision of farmers throughout the year and especially when picking oranges. Indeed, the fight against *Penicillium* rots may be indirect before harvest by controlling the causes that can cause injuries and then the penetration of these fungi or serve as their transmission medium (Brown and Eckert 1988). Similarly, other measures to reduce contaminations at harvest could be practiced like the use of disinfected boxes (Fourtassi 1998). Furthermore, spores of *P. digitatum* and *P. italicum* are present in the air and at ground level in orchards (Eckert 1990), so the collection must be done with great care to avoid any sort of injuries that may be sites of infection for spores of *Penicillium spp.* (Tuset and al. 1981 cited by Loussert 1989). For that, harvesters must use special tongs picking; these tools allow the cut of fruit with the stalk with no risk of injuries (Loussert 1989). In addition, the incidence of infection during and after harvest can be reduced by harvesting in dry weather and eliminating fruit falls or injuries (Fourtassi 1998).

4. Conclusion

To sum up, farmers should undergo more rigorous supervision by experts during the year and the gathering period, on the aim to moderate phytosanitary treatments and avoid MRL excess of malathion, methidathion and dimethoate which are very harmful for the environment and human health and minimize physical damages when picking the fruit by adequate manipulations. In fact, any injury is an entry point for parasitic fungi spores which would evolve into the atmosphere and lead rotting fruit. Therefore, considerable damage can ensue and the value of the crop obtained after much effort could be severely compromised, hence the importance to provide more technical advice for better use of production and maintain the rank of export of the 'Maltaise' orange.

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