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Management of *Ceratitis Capitata* and *Phyllocnistis citrella* with Basalt powder "Farina di Basalto®" compared to two botanical extracts (*Citrus aurantium* and *Nerium oleander*) in citrus orchard

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Abstract
This study aims to evaluate and compare the efficiency of Basalt as foliar spray with two doses (1.5% and 3%), in the ground as a fertilizer (1.5%), and as a combined application (1.5%) compared to two botanical aqueous extracts of bitter orange (*Citrus aurantium*) and *Nerium* (*Nerium oleander*) (50g/l against *Ceratitis Capitata* and *Phyllocnistis citrella* in citrus orchard. Concerning *C. capitata*, results a repellent effect exerted by used products. Trapped adults mean number in all treated units significantly decreased after first treatment and was maintained at very significant low values compared with control. Regarding *P. citrella*, alive larvae population decreased considerably after first treatment and reached low values. Larvae mortality rates in treated plots were observed since the first day after first treatment. They increased lately till reaching mean values ranking between 80 and 100% one and two weeks after second treatment. Efficacy rates of basalt were about 60, 51 and 39% respectively for foliar applications at 3% and 1.5% and the combined application. Regarding plant extracts, they were about 50 and 56% respectively for Nerium and bitter orange. These results will help in planning integrated pest management in citrus orchard to control *C. capitata* and *P. citrella* in order to ovoid use of pesticides.

Keywords: Citrus, medfly, citrus leafminer, biological control, basalte, mortality rates, Farina di Basalto®

1. Introduction
Originated from southeastern Asia, particularly southern China, *Citrus* is a very cultivated crop worldwide [1]. In Tunisia, Citrus is a very important crop with approximately 6.4 million trees covering an area of about 21 000 hectares. Citrus production represents 9.45% of total fruit production. It holds an important position in exportation and an economic role in Tunisia [2, 3]. However, Citrus is attacked by a large number of pests that cause significant losses especially the Mediterranean fruit fly (Medfly) *Ceratitis Capitata* Wiedemann (1824) (Diptera: Tephritidae) and the Citrus Leafminer *Phyllocnistis citrella* Stainton (1856) (Lepidoptera: Gracillariidae) [4]. The Medfly is a serious pest of fruits worldwide [5]. It is a polyphagous pest that may attack and damage a large number of fruits belonging to more than 350 botanical species [6, 7]. *C. capitata* causes damages on commercial and noncommercial fruits and is the most damageable pest of fruit trees in Tunisia especially citrus crop [8, 9]. This fly causes important damages also on peaches and figs (*Ficus indica*) [4]. In Tunisia, the losses reach up to 80% and 90% in the absence of control strategies [4, 10, 11]. This pest, may limit citrus exportation as well as obliging farmers to apply highly disinfection measures [3, 12, 13]. Medfly control in many countries around the world involves use of insecticides such as organophosphates, pyrethroids and spinosad released with protein baits spray [14, 16] or spraying also the soil in order to destroy larvae or pupae essentially [17]. In Tunisia, Medfly control is mainly relied on the application of organophosphate insecticides particularly Malathion merged with protein baits [18]. However, different problems have been associated to the use of organophosphate insecticides mainly the problem of resistance, like for instance to Malathion [19] and negative effect on non-target fauna [20, 21].

Different alternative ways have been applied in Tunisia in order to control *C. capitata* including SIT (Sterile Insect Technique) [22, 23], mass trapping [12, 13, 23] and attract and kill method [9]. The Citrus leafminer (CLM) *P. citrella* damages Citrus spp and some cultivars included in the Rutaceae family [24]. In Tunisia, *P. citrella* was mentioned for the first time in October 1994 in the northeast and had been distributed after in all citrus-cultivating regions [25]. This pest attacks young shoots Adults lay eggs near the leaf midrib and hatched larvae start to feed on leaf mesophyll causing serpentine mines. They prefer the lower surface and destroy the epidermis layer of the leaf which rolls up and becomes necrotic [26, 28]. *P. citrella* populations development coincides with summer and autumn flushes of Citrus depending on the variation of temperature. During winter and beginning of spring, the CLM activity becomes lower. Population becomes higher in summer and autumn flush when the volume of foliage is important and favorable for the development of the pest [29]. *P. citrella* feeding affects photosynthetic activity of leaves and thus the quality of Citrus fruit [30]. In Tunisia, highest population activity of the CLM is observed during autumn but it decreases during winter where no young leaves are present [31]. Under Mediterranean climate, summer and autumn flushes, which represent 20 to 30% of total annual foliage development, are the most infested by *P. citrella* [32]. Since its appearance in Tunisia, several strategies have been occurred to control the CLM especially use of pesticide with a large spectrum of action [33]. The conventional way was the single applied mean to control this pest. Nevertheless, this approach becomes costly and short-termed solution [26, 83, 36]. Recent studies showed some alternatives to chemical control against the CLM such as biopesticide and mineral oil applied in home gardens and orchards [37, 39]. The application of foliar spray of mineral oils has been suggested as repellent for females to lay eggs [40, 41]. Besides, these oils have been proved as eco-friendly because they do not threaten beneficial fauna compared to conventional pesticides. In Tunisia, no studies have been carried out about the effect of biopesticides against *P. citrella* [33].

The aim of this work is to evaluate and compare the effectiveness of two plant extracts (Nerium and bitter orange) and the mineral powder of basalt rock "Farina di Basalto®" as alternative ways to control two main pests of Citrus orchards; the Medfly and the Citrus Leaf miner.

2. Materials and methods

2.1. Experimental site

This work was conducted in a conventional citrus orchard located in the region of Testour (36°33'14.7"N 9°23'40.4"E) (Governorate of Beja) in Tunisia (Figure 1). The experimental site belongs to the Society of Agricultural Development of Ghanima Andalous (SDAE El Ghanima Andalous) in the region of Testour. Navel Fukumoto is the cultivated variety in the citrus orchard in an intensive mode. Choice of the citrus orchard was based on the very important attack of citrus trees by several pests especially the Medfly and the Citrus Leaf Miner in the region of Testour.

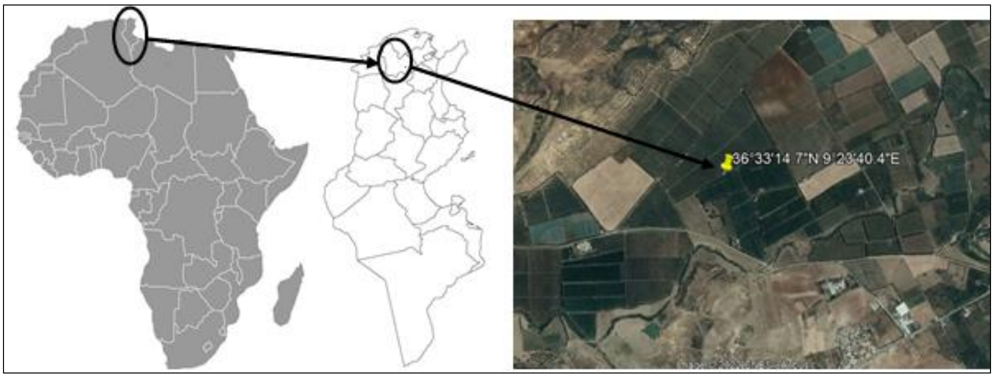

Fig 1: Geographical localization of the experimental site.

2.2. Study period

This study was carried out from July 07th to August 28st 2020.

2.3. Treatments

2.3.1. Basalt powder "Farina di Basalto®"

Known at first as a fertilizer applied to improve crop production, "Farina di Basalto®" contains natural elements such as Silicium, Alumina, Potassium and Calcium. Basalt powder or "Farina di Basalto®" is obtained from Basalti Orvieto s.r.l. from the basaltic effusions in Italy. "Farina di Basalto®" is obtained through an industrial process (patented) of grinding selected basalt rocks and not by ventilation. It is micronized with particles less than 30 µm. These particles are obtained by mechanical grinding of the basic volcanic effusive rock. This product is not harmful for human and ecofriendly and activates and improves plant defense system [42, 45].

The chemical composition of "Farina di Basalto®" samples, consisting of 37.76-59.64 SiO2; 10.10-20.93 Fe2O3; 11.77-14.32 Al2O3; 5.57-14.75 CaO; 5.37 to 9.15 MgO; 1.40-3.34 Na2O [46]. Chemical properties of basalt powder of "Farina di Basalto®" are shown in Table 1 and physical properties in Table 2.

Table 1: Chemical proprieties of basaltic mineral fines of "Farina di Basalto®" [42].

Component	Percentage
(SiO_2)	49%
(Al_2O_3)	20.5%
(K_2O)	8%
(Fe_2O_3)	7.5%
(CaO)	7.2%
(MgO)	2.8%
(Na_2O)	2.5%

Table 2: Physical properties of "Farina di Basalto®" [47].

Properties	Descriptions
Density	Mg / mc 2,70
Solubility	Not soluble in water
Physical state	Solid powder
Color	Slightly gray color
Odor	non perceptible
pH	7+/0.2
Fusion point	1200 °C
Flash point	Not flammable
Explosive properties	Not explosive
Oxidizing properties	No oxidizing properties

2.3.2. Plant extracts

In order to compare with "Farina di Basalto®" impact, two plant extracts were tested in this trial. Fruits of bitter orange were collected from trees of *Citrus aurantium* from the Higher School of Agriculture of Mograne (36°25'45.90''N 10°05'36.55''E) belonging to the Governorate of Zaghouan in Tunisia. Regarding Nerium (*Nerium oleander*), leaves were taken from trees growing in the Higher School of Engineer of Medjez El Beb (36°37'22.13"N 9°33'34.89"E) that belong to the governorate of Beja in Tunisia. In the laboratory orange bitter fruits were peeled and dried at ambient temperature, same as for Nerium leaves, and then grinded to obtain a very fine powder. Extracts were tested at a dose of 50 g of powder dissolved per one liter of water according to previous trials [48, 49]. Powders were mixed with water during 48 hours of maceration, then filtered with muslin and pulverized as foliar application using a motorized backpack atomizer.

2.4. Experimental design

The experimental design is a complete random block (CRB) where seven treatments have been applied: (C) as control, (XF 1.5%) for foliar "Farina di Basalto®" application at a dose of 1.5%, (XF 3%) for foliar basalt application at 3%, (FP 1.5%) for "Farina di Basalto®" applied as a fertilizer in the ground at 1.5%, (XF+FP 1.5%) for combined application at 1.5%, (Org) for orange bitter extract applied at a dose of 50 g/l and (Ner) for Nerium extract applied at a dose of 50 g/l (Table 3). Three lines in the citrus orchard were adopted for this trial and for each treatment three trees were chosen randomly (Figure 2).

Table 3: Applied treatments with doses and abbreviations

Treatment	Abbreviation	Dose
Control	C	None
Foliar "Farina di Basalto®" application at 1.5%	XF 1.5%	1.5%
Foliar "Farina di Basalto®" application at 3%	XF 3%	3%
"Farina di Basalto®" as a fertilizer at 1.5%	FP 1.5%	1.5%
Combined application 1.5%	XF+FP 1.5%	1.5%
Orange bitter extract	Org	50 g/l
Nerium extract	Ner	50 g/l

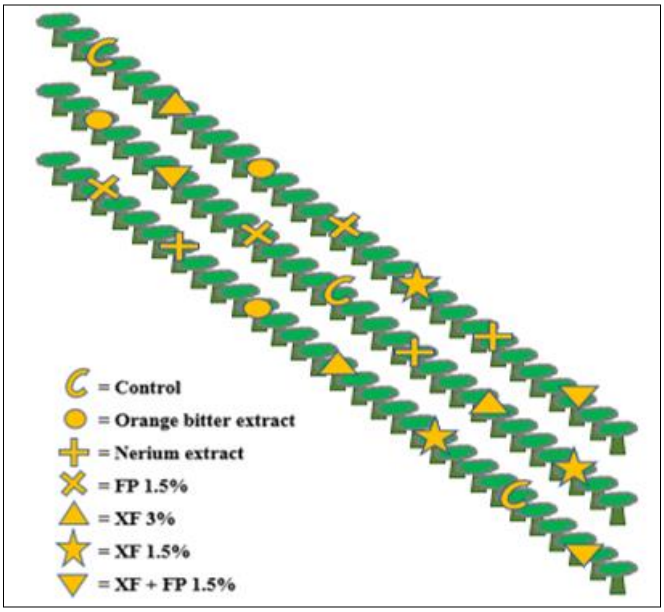

Fig 2: Set up of the experimental design (C: Control, XF3%: "Farina di Basalto®" foliar application at 3%, XF1.5%: "Farina di Basalto®" foliar application at 1.5%, FP1.5%: "Farina di Basalto®" application in the ground, XF+FP1.5%: Combined "Farina di Basalto®" application).

2.5. Sampling and monitoring

Sampling started one week before first treatment. Then, it took place during the treatment day, after 24 hours, 72 hours, one week and then two weeks after first treatment. Same sampling interval time was adopted after the second treatment. From each tree, four twigs of about 30 cm of length were sampled from each side (north, east, south, west). Twigs were placed into black plastic bags that were marked and then taken to the laboratory for monitoring. Regarding Medfly monitoring, Mcphail-type traps were installed in each randomly chosen citrus tree. Traps contain a kill-disc with an insecticide, a sexual pheromone capsule "Trimedlure" and protein-based bait.

2.6. Efficacy evaluation

Where Mr is the mortality rate, Mt is the number of dead individuals in treated plots, Mc is the number of dead individuals in untreated plots (Control).

Regarding the efficacy rate, it was evaluated according to the following formula (Abbott 1925) [50]:

$$Er = [(T0 - Tt / T0) \times 100]$$

Where Er is the percentage of efficacy, T0 is number of living individuals on untreated plots, Tt is number of living individuals on treated plots.

2.7. Identification of phenolic compounds by HPLC-PDA/ESI-MS analysis

In order to identify phenolic compounds, 20 g of dried leaves were macerated in a volume of 100 mL of distilled water during a period of 48 h at room temperature. Obtained solutions were filtered, lyophilized and then transferred to vials and stored at 4 °C temperature in the dark. Phenolic compounds were identified using high performance liquid chromatography coupled with a photodiode array and mass spectrometry detection (HPLC-PDA-ESI/MS). LC–ESI–MS analysis was conducted in negative electrospray ionization mode on an Agilent 1100 series HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a photodiode array detector (PDA) and a triple quadrupole mass spectrometer type Micromass Autospec Ultima Pt (Kelso, UK). Analysis was run on a reversed-phase Uptisphere C18 (Interchim) (2 mm × 100 mm, 5 µm particle size) at 40 °C. The mobile phase for the separation of extracts' compounds was composed of water, 0.1% formic acid in water (A), and 0.1% formic acid in methanol (B). The following multi-step linear solvent gradient was employed: 0-5 min, 2% B; 5-60 min, 98% B, 60-65 min, 2% B.

2.7. Statistical analysis

Statistical analysis were performed using the statistical software program SPSS 23 (Statistical Package for the Social Sciences version 23). This program was used for analysis of variance (ANOVA) and LSD test for mean comparison at $p \leq 0.05$.

3. Results

3.1. HPLC-PDA/ESI-MS analysis

The HPLC-PDA/ESI-MS analysis permitted to identify in *C. aurantium* extracts seven flavonoids among which five flavonones which are Eriodictyol, Naringenin-rutinoside, Eriocitrin, Brutierdin and Hesperidin which was the dominant compound, one flavonol which is the Quercetin and one Limonoid (Limonin). Regarding *N. oleander* extract, six compounds were identified; Quinic acid, Caffeoylquinic acid Isomer I, Caffeoylquinic acid Isomer II, Kaempferol-rutinoside, Dicaffeoylquinic acid and Quercetin rutinoside (rutin) that was the dominant compound.

3.2. Treatments' impact on the CLM

Monitoring alive CLM larvae in leaves (Figure 3) showed that one day after first treatment (T1+1 day) mean values in all treated plots started to decrease and were significantly different compared to control ($p \leq 0.05$). This decline was continuous even after second treatment (T2) till reaching very low values approaching zero at the end of the study period while in control plots mean values were about 5 alive CLM larvae per sample. It must be noted that no significant differences were noted between different types of application ("Farina di Basalto®" and plants extracts) except for combined one (XF+FP1.5%).

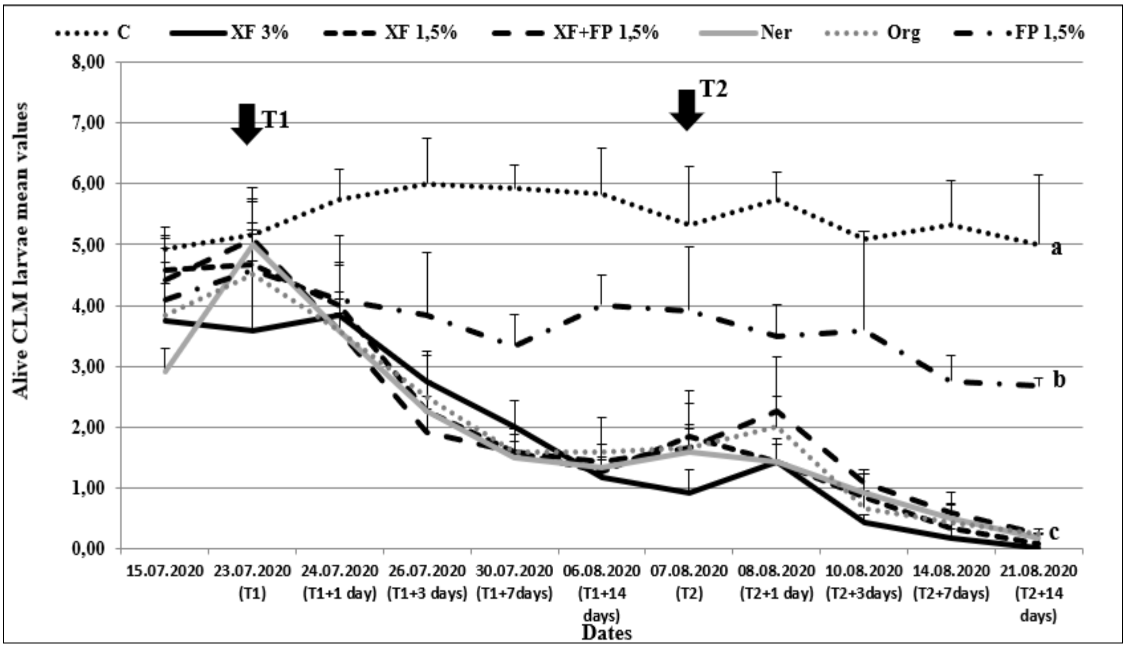

Fig 3: Monitoring alive CLM larvae mean values in leaves in relation with the different treatments in the citrus orchard (Legend: C: Control, XF3%: "Farina di Basalto®" foliar application at 3%, XF1.5%: "Farina di Basalto®" foliar application at 1.5%, FP1.5%: "Farina di Basalto®" application in the ground, XF+FP1.5%: Combined "Farina di Basalto®" application, Ner: Nerium, Org: Bitter orange, T1: First treatment, T2: Second treatment) (Means with the same letters are not significantly different at $p \leq 0.05$).

Regarding mortality rates (Figure 4), obtained results showed a quick effect on larvae one day after first treatment (T1+1 day) where maxima with no significant differences ($p \leq 0.05$) were observed for XF3%, XF+FP1.5% and Orange bitter with respectively 14.89, 14.26 and 14.23%. Mortality rates increased then to reach average values of about 47.16 and 49.42% respectively for XF3% and Orange bitter two weeks after first treatment. However, FP1.5% where "Farina di Basalto®" was applied in the ground as a fertilizer did not show high mortality rates and was always significantly different (p≤0.05) from the others. It must be noted that after the second treatment (T2), mortality rates continued to increase till reaching average values between 90.46 and 100% for XF3%, 80.87 and 92.38 for XF1.5%, 72.80 and 90.16% for Nerium and 8.41 and 86.86% for orange bitter respectively one week and two weeks after T2 and mostly with no significant differences between treatment (p≤0.05). In fact, these fundings indicate that complementary application is needed to strengthen the effectiveness of the first one.

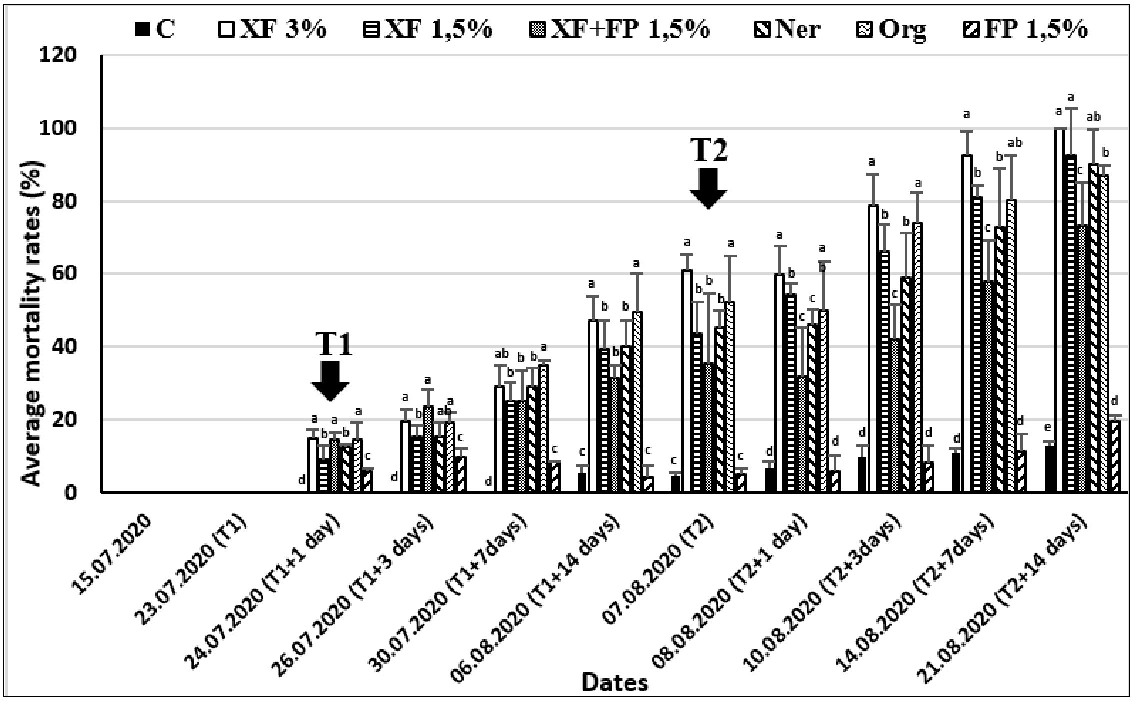

Fig 4: Mortality rates mean values monitoring of CLM larvae in leaves for the different treatments in the citrus orchard (Legend: C: Control, XF3%: "Farina di Basalto®" foliar application at 3%, XF1.5%: "Farina di Basalto®" foliar application at 1.5%, FP1.5%: "Farina di Basalto®" application in the ground, XF+FP1.5%: Combined "Farina di Basalto®" application, Ner: Nerium, Org: Bitter orange, T1: First treatment, T2: Second treatment) (Means with the same letters are not significantly different at *p*≤0.05).

Efficacy rates of all used products with different doses and applications were not significantly different (p≤0.05) except for FP1.5% where it was about 9% only. Most important efficacy rate was observed for XF3% followed by orange bitter, XF1.5%, Nerium and XF+FP 1.5% with respectively 60, 56, 51, 50 and 39% (Figure 5). In fact, these findings prove that different types of applications are statistically equal and similar.

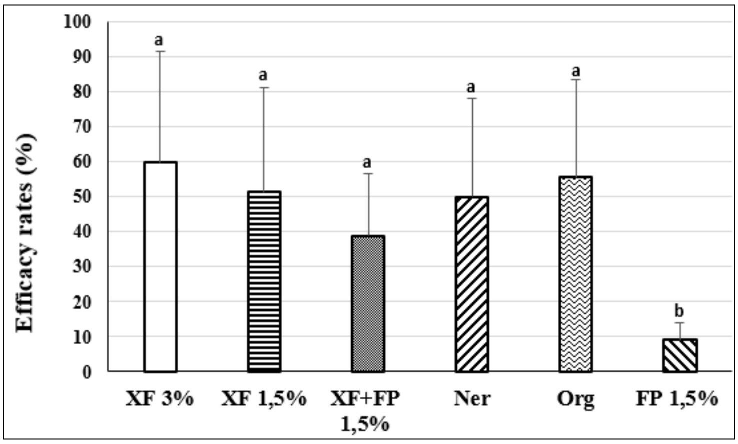

Fig 5: Efficacy rates of different treatments used against CLM larvae in the citrus orchard. (Legend: C: Control, XF3%: "Farina di Basalto®" foliar application at 3%, XF1.5%: "Farina di Basalto®" foliar application at 1.5%, FP1.5%: "Farina di Basalto®" application in the ground, XF+FP1.5%: Combined "Farina di Basalto®" application, Ner: Nerium, Org: Bitter orange) (Means with the same letters are not significantly different at p≤0.05).

3.3. Treatments' impact on Medfly adults

Monitoring Medfly adults in the citrus orchards showed a very important infestation due to the high adults' number encountered in installed traps comprised between a minimum of 7.33 and a maximum of 29.66 adults per trap one week before first treatment and between 8 and 44.66

adults per trap during the treatment's day (Figure 6). One day after first treatment, mean values of trapped adults decreased in all treated plots with no significant differences ($p \leq 0.05$). These averages did not show important variations during the rest of the study period and was maintained between 5 and 9 adults per trap. One week and then two weeks after the second treatment, adults mean values was very low in all treated plots. Regarding FP1.5%, adults population decreased slightly to resume its increase later approaching mean values observed in control plots especially after one day after second treatment where no significant difference was observed between FP1.5% and control.

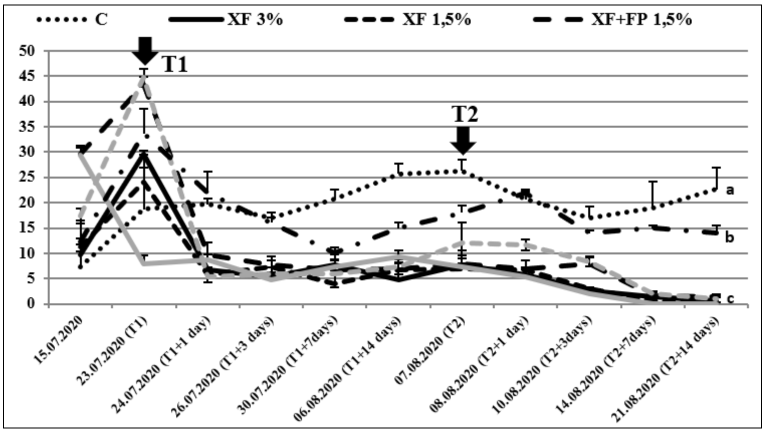

Fig 6: Monitoring trapped Medfly adults mean values in relation with the different treatments in the citrus orchard (Legend: C: Control, XF3%: "Farina di Basalto®" foliar application at 3%, XF1.5%: "Farina di Basalto®" foliar application at 1.5%, FP1.5%: "Farina di Basalto®" application in the ground, XF+FP1.5%: Combined "Farina di Basalto®" application, Ner: Nerium, Org: Bitter orange, T1: First treatment, T2: Second treatment) (Means with the same letters are not significantly different at $p \leq 0.05$).

4. Discussion

Obtained results during this study showed that biological control against Medfly and the CLM in citrus orchard using "Farina di Basalto®" and plant extracts was effective as an alternative way in an IPM approach. In fact, used methods were effective to reduce pest populations levels to low values under harmful threshold same as using insecticide sprays or mass trapping technique to control Medfly in the Mediterranean [9, 13, 51] or pesticides and mineral oils for the CLM [33]. However, use of pesticides or mass trapping technique seems to be costly due to the high costs of traps and their attractants [52]. On the other hand, use of alternatives against *C. capitata* and *P. citrella* is needed due to the different major issues such as problems of resistance of the Medfly [53, 56] and the CLM. Further, Hafsi *et al.* [9] confirmed the ineffectiveness of insecticides in the limitation of Medfly resistance. In Tunisia, *C. capitata* is controlled by the mass trapping technique with different types of traps and baits as an alternative way of control [57, 58]. On Citrus orchards Hafsi *et al.* [23] demonstrated the efficacy of a density of 80traps/ha in reducing population level and damaged fruits. On apricots, Elimem *et al.* [13] demonstrated that 40 traps/ha density is efficient in reducing population in the field. This is probably influenced mainly by the degree of isolation, the size of the protected orchard and essentially by pest population density [59]. Regarding *P. citrella*, different methods have been proved as alternatives such as cultural technique consisting in enhancing the spring flushes which is unfavorable for the pest to reduce or to limit the outbreaks of CLM during summer and autumn flushes [60]. Chemical pesticides and mineral oils are the most used methods to control CLM [10, 33]. Obtained results showed the effectiveness of basalt powder and plant extract in reducing both pests' populations. The mineral product (basalt powder) showed the highest mortality rates for the CLM at 3%. Same results were demonstrated in other studies were basalt reduced many other pests' populations such as the western flower thrips *Frankliniella occidentalis* Pergande (1895) (Thysanoptera, Thripidae) and the broad mite *Polyphagotarsonemus latus* Banks (1904) (Acari, Tarsonemidae) in protected crops under greenhouses [43], the red flour beetle *Tribolium castaneum* Herbst (1797) (Coleoptera, Tenebrionidae), the lesser grain borer *Rhyzopertha dominica* Fabricius (1792) (Coleoptera, Bostrichidae) and the Mediterranean flour moth *Ephestia kuehniella* Zeller (1879) (Lepidoptera, Pyralidae) on stored wheat [44]. Regarding the Medfly, use of basalt powder reduced adults' number in installed traps to low values compared with control [61] confirming thus results found during this study. In fact, inert powders or dusts have been used as alternatives for centuries in the control of insect pests. These powders contain a wide range of minerals such as silica [62, 63]. These products have been used to control several pests, more particularly insects in stored foodstuffs [64, 65]. In fact, they act as absorbents of the waxy layer of the exoskeleton of insects and arthropods in general causing the death of the animal by desiccation [62]. In the same context, Silica or Silicon dioxide (SiO_2), which is the most important component of "Farina di Basalto®" with 49% as indicated in table 1 has an important role in reducing several insect pests populations [66] as this compound is responsible for the desiccation of insects [64] which leads to insect mortality. These results were indicated with other natural silicious sedimentary rock such as Diatomaceous Earth (80 to 90% of Silica) which has an ovicidal potential on the carob moth *Ectomyelois ceratoniae* Zeller (1839) (Lepidoptera; Pyralidae) with an emergence rate not exceeding 20% [67]. On the other hand, Kaolin, rich in Aluminum Silicate (Al_2SIO_5), has been proved to be efficient to control *P. citrella* in reducing pest population in citrus orchard [33]. These different types of inert powders are used to control protected stored grains [68], with an insecticidal and repellent effect on pests during storage, in greenhouses and in fields [43, 44, 62, 67]. In the same context, "Farina di Basalto®" has been proved as a repellent for thrips species such as *F. occidentalis* under greenhouses [43] and for *C. capitata* in pomegranate orchard [61], where treated plots did not attract pests anymore.

Furthermore, botanical extracts (Nerium and orange bitter) showed important impacts on both pest species in citrus orchards. Several studies demonstrated insecticidal and repellent effects of *Citrus aurantium* against several pests such as *C. capitata* and *Bactrocera oleae* Rossi (1790) (Diptera, Tephritidae) [69], *Spodoptera frugiperda* Smith (1797) (Lepidoptera: Noctuidae) [70] stored products insects with anti-acetylcholinesterase activity [71] and mealybugs [72]. The flavedo of common bitter contains secondary metabolites with insecticidal activity [69]. In addition, essential oils of *C. aurantium* have shown insecticidal activity against *C. capitata* [73] as well as lepidopteran pests [74]. Same as for Nerium, it has been proved that it has an insecticidal and even bactericidal effect [75], and causes mortality of many insect pests such as the bean weevil *Acanthoscelides obtectus* Say (1831) (Coleoptera, Bruchidae) [76], *Paederus fuscipes* Curtis 1826 (Coleoptera, Staphylinidae) [77] and the processionary moth *Thaumetopoea wilkinsoni* Tam (1924) (Lepidoptera, Notodontidae) [78]. The most detected toxic glycosides in common oleander are oleandrine, oleandrigenin, digitoxigenin, nerine, folinerin and rosagenin [79, 80].

Two other fundings were demonstrated during this study. In fact, secondary applications of “Farina di Basalto®” as well as for both plant extracts are needed to enhance mortality rates of the CLM and to maintain trapped adults’ populations of the Medfly at very low values. In fact, Elimem and Chermiti [81] confirmed the necessity of complementary treatments to enhance the first treatment and increase mortality rates for bio-pesticides. On the other hand, “Farina di Basalto®” when used as foliar application (XF3% and XF1.5%) has a significant impact on both pest species than used as as a fertilizer in the ground (FP1.5%). “Farina di Basalto®” aerial sprays directly influence the dynamics of pest populations and significantly reduces their number [82].

5. Conclusion

To substitute chemical insecticides, biological control seems to be an alternative way to control sever pests in citrus orchards including the Citrus Leaf Miner (CLM) *Phyllocnistis citrella* and the Mediterranean fruit fly (Medfly) *Ceratitis Capitata*. Use of “Farina di Basalto®” a unique basalt powder and plant extracts; bitter orange (*Citrus aurantium*) and Nerium (*Nerium oleander*), with different doses showed that Medfly adults’ population decreased in all treated plots showing thus the insecticidal and repellent impact of used products. Regarding the citrus leaf miner, alive larvae populations decreased considerably compared with control and mortality rates increased since first day after first treatment and continued after second application till reaching values approaching 100%. These findings may be considered as a promising biological agent in an Integrated Pest Management.

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