Behavioral response of *Panonychus citri* (McGregor) (Acari: Tetranychidae) to synthetic chemicals and oils (#52647)

First revision

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Behavioral response of *Panonychus citri* (McGregor) (Acari: Tetranychidae) to synthetic chemicals and oils

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Background *Panonychus citri* (McGregor) (Acari: Tetranychidae) population outbreaks after the chemical application on the citrus plantation is a common observation. To understand the field outbreak of *P. citri*, dispersal behavior can be better understood. Therefore, in the current study, the dispersal activity of *P. citri* was observed on the leaf surfaces of *Citrus rticulata* (Rutaceae) treated with SYP-9625, abamectin, vegetable oil, and EnSpray 99. Water was used as a control treatment. Method Mites were released on the first (apex) leaf of the plant (adaxial surface) and data were recorded after 24 h. The data of treated, un-treated, and half-treated were combined for leaf surfaces (adaxial right, adaxial left, abaxial right and abaxial left) further analysis. All experiments were performed in the open-air environmental conditions. Results The maximum number of mites was captured on the un-treated or half-treated surfaces due to the repellency of chemicals. By comparing the treated and untreated surfaces, significant differences were observed: within treated and un-treated surfaces adaxial right at LC_{30} (sub-lethal) and LC_{50} (lethal), within treated surfaces of the adaxial left at LC_{50}, within treated and un-treated of abaxial right at LC_{50}, and within treated of the abaxial left at LC_{30} and LC_{50} doses among the treatments. During treated and half-treated experimentation, non-significant and significant differences were observed at LC_{30} and LC_{50} respectively, on the adaxial and abaxial surfaces. Mites captured among the treatments were found significantly different on the abaxial surfaces at LC_{30} and no difference was observed at LC_{50} by treating the whole plant. Therefore, the presence of tested acaricides indeed interferes with *P. citri* dispersal within leaf surfaces of plantations depending on the mites released point as well as a preferred site for feeding.

Behavioral response of *Panonychus citri* (McGregor) (Acari: Tetranychidae) to synthetic chemicals and oils

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Abstract

Background

Panonychus citri (McGregor) (Acari: Tetranychidae) population outbreaks after the citrus plantation's chemical application is a common observation. Dispersal behavior is an essential tool to understand the secondary outbreak of *P. citri* population. Therefore, in the current study, the dispersal activity of *P. citri* was observed on the leaf surfaces of *Citrus reticulata* (Rutaceae) treated with SYP-9625, abamectin, vegetable oil, and EnSpray 99.

Method

Mites were released on the first (apex) leaf of the plant (adaxial surface) and data were recorded after 24 h. The treated, untreated, and half-treated data were analyzed by combining the leaf surfaces (adaxial right, adaxial left, abaxial right and abaxial left). All experiments were performed in open-air environmental conditions.

Results

The maximum number of mites was captured on the un-treated or half-treated surfaces due to chemicals repellency. Chemical bioassays of free-choice teste showed that all treatments significantly increased the mortality of *P. citri* depending on application method and concentration. A significant number of mites repelled away from treated surfaces and within

treated surfaces except adaxial left and abaxial right surfaces at LC_{30}. In the no-choice test, SYP-9625 gave maximum mortality and dispersal by oils than others. No significant differences were observed within adaxial and abaxial except abaxial at LC_{30}. Therefore, the presence of tested acaricides indeed interferes with *P. citri* dispersal within leaf surfaces of plantations depending on the mites released point and a preferred site for feeding.

Introduction

The citrus red mite, *Panonychus citri,* is a serious pest of the citrus growing region all over the world (Gotoh & Kubota, 1997; Kasap, 2009; Faez et al., 2018b; Korhayli et al., 2018) as well as from China (Yuan et al., 2010; Fang et al., 2013; Liu et al., 2019). The southern region of China, known as *P. citri,* preferred area due to favorable climatic conditions (Shi & Feng, 2006). The immature and adult stages feed on leaves and fruit by giving stippling damage, which inhibits the photosynthesis process and leads shoot dieback and leaf/fruit dropping (Kranz et al., 1978). Server infestation in the field may cause irritation and allergic reactions to citrus workers (Fernández-Caldas et al., 2014).

The chemicals application is a preferred method to control *P. citri* by the farmers in citrus orchards (Gotoh & Kubota, 1997; Chen et al., 2009; Kasap, 2009; Fadamiro et al., 2013; Faez et al., 2018a,b; Karmakar, 2019; Liu et al., 2019). SYP-9625 and abamectin are commonly used among synthetic chemicals against citrus pests in China (Gu et al., 2010; Hu et al., 2010; Liu et al., 2018; Chen et al., 2019). It is essential to find alternative products (Isman, 2008; Tak & Isman, 2017) for synthetic chemicals due to serious threats for non-target organisms and the environment (Kumral et al., 2010; Chen & Dai, 2015). Agricultural mineral oils (EnSpray 99) are compatible with predatory mites and effective against horticultural crops pests (Wang et al., 2004; Chen & Zhan, 2007; Xue et al., 2009a,b; Teifemg et al., 2011; Zhuang et al., 2015). Vegetable oils are also considered an alternative due to toxicity and repellency against target pests widely reported (Koulbanis et al., 1984; Ismail et al., 2011; Oliveira et al., 2017). Vegetable oil extracted from kitchen/household waste (vegetable remaining) were used in this study. Guangdong Institute of Applied Biological Resources, China, provided this kitchen vegetable waste oil (trial product).

Environmental contamination such as pesticides can influence mites behavioral activities on leaves or plants (Ibrahim & Yee, 2009; Lima et al., 2013; Cordeiro et al., 2014; Monteiro et al., 2019a). The behavioral changes due to chemicals affect pest management (Guedes et al., 2016). The population outbreaks of plant-feeding mites after the chemical application on the horticultural crops are prevalent (AIiNiazee & Cranham, 1980; Zwick & Field, 1987). The abrupt increase of the mites population has many suggestions by the researchers; the most critical explanation suggests the impact of chemicals on the natural enemies (AIiNiazee & Cranham, 1980; Dittrich et al., 1980; Zwick & Field, 1987). Iftner & Hall (1983) reported that increasing the chemical application rates in the absence of natural enemies also increases pest numbers. Therefore, many factors are involved. Since, agrochemical impact on target pest or insect can be assessed through the application rate (lethal and sublethal), application timing, and mode of

action. The use of the sublethal effect of chemicals is considered a more accurate approach to measure toxicity, which changes individuals behavioral responses that survive from toxic exposure (Desneux et al., 2007; Biondi et al., 2013; Turchen et al., 2016; Alves et al., 2018). Dispersal behaviors define as any movement from one place to another for the survival of any organism due to environmental stress or non-viable to live (e.g., lack of food or surrounding climatic constraints) (Clobert et al., 2001; Ims & Hjermann, 2001). Dispersal movement done in three stages; emigration, a vagrant stage, and immigration (Ronce, 2007), which depend on the species life cycle, sex, environmental variations, space, and time (Dunning Jr et al., 1995; Hanski, 1998, 1999; Turchin, 1998; Bergman et al., 2000; Bowler & Benton, 2005).
The dispersal behavior of mites uses active or passive dispersal mechanisms (Evans, 1992; Sabelis & Afman, 1994; Tixier et al., 1998; Perotti & Braig, 2009). Active dispersal (walking) is the most preferred mechanism in mites due to morphological characteristics and short-range travel (Strong et al., 1999; Melo et al., 2014; Monteiro et al., 2019a). Like most of the tetranychids, *Panonychus citri* also do passive dispersal by silk threads (aerial dispersal) to overcome crowding, food depletion (Bell et al., 2005), and light-dependent (Pralavorio et al., 1989). In this study, we evaluated the lethal and sublethal effects of selected pesticides on the dispersal pattern of *P. citri* by treating the leaf surfaces. We hypothesized that *P. citri* response towards chemicals treatment may ~~or may not~~ reason for the population outbreak in the field conditions.

Materials & Methods

Mite Culture

Mite culture was regularly maintained since 2019, on lemon leaves with the water-saturated sponge. The culture was reared in the growth chamber with 16:8h (Light: Dark) photoperiod and 26±1 ºC temperature. One to three-day-old adult females (He et al., 2011; Alves et al., 2018) ~~was~~ used for said experimentation reared in the laboratory for several generations (more than 50 generations). The mite culture was shifted to the open-air environment one month before the experiment to acclimatize ~~to that environment~~.

Plants

Citrus plants (*Citrus reticulata*) approximately 1-2 months old were used after shifting to the pots. The plants with 7 to 8 leaves were used by leaving six leaves (3 on the right and left side) and cutting them. All plants were washed three times with water to be sure not to have any arthropods on it. The bottom of each plant stem was wrapped with wet tissue paper and maintained wet to keep mites on the plant. All plants were manured and watered accordingly under reasonable conditions during January.

Chemicals

SYP-9625 30% EC and Abamectin 5%EC, EnSpray 99% EC (EnSpray 99), and ~~V~~egetable oil 99% were used in this research. Chemicals and EnSpray were bought from the local market. The degummed Vegetable oil was extracted from household daily kitchen vegetable waste. ~~The degumming method was carried out at 60-70 ºC with 1-3% water and 20-30 minutes stirring. The~~

precipitates formed during this process were removed by centrifugation. Vegetable oil was provided by the Guangdong Institute of Applied Biological Resources.

Each chemical toxicity was calculated using a modified leaf dip bioassay (Wang et al., 1971; Nauen, 2005) previously in the laboratory. The selection concentrations of each chemical were made with 90 to 10% corrected mortality after 24 h. Lethal and sublethal concentrations of each chemical were calculated by probit analysis with SPSS version 22.0 software (Weinberg & Abramowitz, 2016). In this experiment, we used LC_{30} (0.065%, 0.049%, 0.024% and 0.08%) and LC_{50} (0.196%, 0.110%, 0.051% and 0.024%) for SYP-9625, Abamectin, Vegetable oil and EnSpray 99, respectively.

Experimental methodology

The method adopted by Iftner and Hall (1983) was followed for the current experiment. Letters were assigned to leaves surfaces as; adaxial right (ADR), adaxial left (ADL), abaxial right (ABR), and abaxial left (ABL). We used a free choice and no choice method by dividing it into nine small experiments, as shown in Fig. 1. Chemicals were applied to the treated leaf surface with a hand sprayer. Untreated part of leaflet or surfaces was protected from chemicals spraying by cardboard shield and plastic bags. Each plant's ground surface was covered with plastic with double side sticky tape on edge. The right adaxial surface was selected for easy to release mites (20 mites x 3 surfaces) and identified mites location from the inoculated surface after 30 minutes of chemicals application. Mites were captured 24 hours by location as per the experimental layout. The mites on the leaf surface, wet tissue paper (chemical treated) and plastic cover (chemical sprayed) were considered as dead. The mites not found as live or dead were considered missing mites. The experiments were used with three replications.

The treated, un-treated, and half-treated data were combined for leaf surfaces (ADR, ADL, ABAR, and ABL) further analysis. All experiments were performed in open-air environmental conditions.

Statistical analysis

The mean number of mites (LC_{30} vs LC_{50}, Treated vs Un-treated, Treated vs Half-treated, and Adaxial vs Abaxial) were analyzed using an independent sample t-test. The difference between control and treatments captured mites means were analyzed using the general linear model (GLM) for ANOVA with Tukey's HSD test (P<0.05). All statistical analysis procedures were calculated with Minitab® 17.3.1 version (Minitab, 2016). Graphical representation was done using GraphPad prism®(Motulsky, 2007) and OriginPro (Edwards, 2002).

A correlation analysis was conducted by comparing toxicity (% mortality) and % mites present on treated, un-treated, and half-treated surfaces to better understand the relationship between the behavioral responses of *P. citri*. Pearson correlation (*r*) and calculating *t* distribution value formulas were used in R.

$$r = \frac{\sum (x - mx)(y - my)}{\sqrt{\sum (x - mx)^2 \sum (y - my)^2}}$$

$$t = \frac{r}{\sqrt{1-r^2}}\sqrt{n-2}$$

n is the length of factor (*df* = n-2) in two vectors (***x*** (toxicity) and ***y*** (mites observed on treated or untreated or half treated surfaces) while ***mx*** and ***my*** are the means of vectors. The significant level can be determined on the *t*-value.

Results

Toxicity

Compared to control, acute toxicity of treatments was found significantly different within each dose in all experiments except in exp. no. 8 at LC_{30}. There was significant difference between doses within abamectin (For exp. no. 3; $t_{-5.56}$=-5.00; *P*= 0.007), SYP-9625 (For exp. no. 4; $t_{-7.78}$=-3.50; *P*= 0.025) and EnSpray (For exp. no. 4; $t_{-8.89}$=-8; *P*= 0.001, For exp. no. 6; $t_{-6.67}$=-3.464; *P*= 0.026) and vegetable oil (For exp. no. 8; $t_{-4.44}$=-2.828; *P*= 0.047) than others. Differences in toxicity (from LC_{30} to LC_{50}) of chemicals to adult (female) *P. citri* occurred among experiments depending on application methods, with ranges in SYP-9625, abamectin, vegetable oil and EnSpray of 1.156 – 2.399 fold, 1.33 – 5.556 fold, 1.249 – 5.005 fold and 0 – 8.889 fold, respectively. Maximum toxicity (%) was observed in the no-choice experiment (the whole plant treated – exp. no. 9), and SYP-9625 more toxic (except in exp. no. 2) than others in all experiments (Table 1).

Re-captured ~~of~~ *Panonychus citri*

According to Fig. 1, the experimental layout is further divided into three parts; Treated vs Untreated (Experiments 1-6), Treated vs Half-treated (Experiments 7-8), and the whole plant treated (Adaxial vs Abaxial) (Experiment 9).

Mites dispersal within the ADR surface were observed 40 to 82.24% (LC_{30}) and 53.7 to 94.067% (LC_{50}) from treated to untreated. The difference between treated and untreated was significantly recognizable. A significant difference was observed in all treatments between the mean number of mites captured on the treated and un-treated on the ADR: SYP-9625 ($t_{-9.22}$ = -5.56; *P* = 0.000), Abamectin ($t_{-7.667}$ = -8.37; *P* = 0.000), vegetable oil ($t_{-10.78}$ = -9.17; *P* = 0.000) and EnSpray 99 ($t_{-8.11}$ = -5.78; *P* = 0.000) except control ($t_{-8.11}$ = -5.78; *P* = 0.097), ~~under~~ LC_{30} while similar results found by applying the LC_{50} doses. The number of mites captured on the treated ADR surface was lower than the number of mites captured on the untreated surface. A maximum number of mites were observed under the un-treated ADR surface at LC_{30} dose of vegetable oil than in the others (Fig. 2).

On the Adaxial surface of left side (ADL), a significant difference was observed within all treatments between the mites captured on the treated and un-treated ADL surfaces: control ($t_{-2.778}$ = -2.94; *P* = 0.015 and $t_{-2.778}$ = -2.94; *P* = 0.015), SYP-9625 (t_{-6} = -3.45; *P* = 0.006 and $t_{-10.22}$ = -8.72; *P* = 0.000), Abamectin ($t_{-5.778}$ = -6.28; *P* = 0.000 and $t_{-7.78}$ = -5.29; *P* = 0.001), vegetable oil ($t_{-4.22}$ = -2.37; *P* = 0.042 and $t_{-4.67}$ = -3.78; *P* = 0.004) and EnSpray 99 ($t_{-4.33}$ = -3.99; *P* = 0.002 and $t_{-9.11}$ = -4.77; *P* = 0.001) on the LC_{30} and LC_{50} doses respectively. A higher number of mites captured on the un-treated surface at LC_{50} of SYP-9625 than others (Fig. 3).

The *Panonychus citri* less visited the abaxial surface than the adaxial surface, so a small number of mites (Mean±SE) were captured but enough for the difference between treated and untreated surfaces. The data collected from abaxial surface of right side (ABR) was ~~observed~~ significantly difference~~ between~~ treated and untreated surfaces: Abamectin ($t_{-1.889}$ = -6.8; P = 0.000), EnSpray 99 ($t_{-1.889}$ = -3.3; P = 0.005) and control ($t_{-1.444}$ = -2.25; P = 0.041), than others at LC_{30} doses while SYP-9625, abamectin and EnSpray 99 were ~~unable to run *t*-test due~~ similar ~~trend~~ between the replication within treated or un-treated and vegetable oil found significantly ~~differ~~ ($t_{-3.222}$ = -4.24; P = 0.002) at LC_{50} doses. The number of mites found maximum on the un-treated ABR surface ~~of abamectin LC_{50}~~ (6.78±0.813) (Fig. 4).

The difference between treated and un-treated was observed significant within all treatments: SYP-9625 (t_{-2} = -4.1; P = 0.003), abamectin ($t_{-3.11}$ = -4.37; P = 0.002), vegetable oil (t_{-2} = -4.94; P = 0.001) and EnSpray 99 ($t_{-2.33}$ = -3.61; P = 0.006) except control at LC_{30} doses on the abaxial surface of left side (ABL). No mites were observed ~~by treating the~~ LC_{50} doses on ABL except vegetable oil ($t_{-3.33}$ = -4.87; P = 0.001) and control (non-significant). Maximum number of mites found on un-treated surfaces depending on the concentration of chemicals (Fig. 5).

On the adaxial surfaces, difference between treated and half-treated surfaces were found similar (non-significant) at LC_{30} except on vegetable oil application ($t_{4.33}$ = 2.8, P = 0.038) while at LC_{50}, all treatments found significant different (For SYP-9625: $t_{8.5}$ = 8.77, P = 0.000; abamectin: $t_{9.167}$ = 10.51, P = 0.000; vegetable oil: $t_{6.167}$ = 9.43, P = 0.000; EnSpray: $t_{8.5}$ = 7.54, P = 0.001) (Fig. 6).

On the abaxial surfaces between treated and half-treated ~~found significantly difference~~ at LC_{30}: abamectin ($t_{2.167}$ = 2.89; P = 0.023), vegetable oil ($t_{2.67}$ = 2.42; P = 0.038) and EnSpray 99 ($t_{3.17}$ = 3.03; P = 0.014) except SYP-9625 and control. At LC_{50}, a significant difference was observed between treated and half-treated surfaces with all mites repelled from treated surfaces (SYP-9625, abamectin and EnSpray 99) (Fig. 7).

In no choice teste (whole plant treated), a significant difference was observed within all treatments (between adaxial and abaxial surfaces): SYP-9625 (t_{-4} = -6.71; P = 0.001), Abamectin ($t_{-3.17}$ = -2.53; P = 0.035), vegetable oil (t_{-8} = -5.37; P = 0.003) and EnSpray 99 ($t_{-7.67}$ = -3.04; P = 0.029) except control ($t_{-1.33}$ = -1.15; P = 0.285) at LC_{30} doses while all treatments found no difference between adaxial and abaxial surfaces at LC_{50} doses (Fig. 8).

Correlation analysis

The correlation between toxicity vs treated and toxicity vs un-treated on both surfaces, either right or left, were found negatively correlated except EnSpray and abamectin (Toxicity vs Treated) at LC_{30} and LC_{50}, respectively (Supplementary Table 1).

The relationship between toxicity and treated surfaces was positively correlated at LC_{30} on adaxial (SYP-9625, abamectin, and EnSpray) and abaxial surfaces (abamectin and EnSpray). There was a significant correlation between toxicity and sublethal half-treated abaxial surfaces of SYP-9625, abamectin, and EnSpray 99. There was a positive correlation between toxicity and lethal half-treated adaxial surface for vegetable oil and EnSpray 99. In contrast, on the abaxial surface, only SYP-9625 found positively correlated (Supplementary Table 2). In a no-choice experiment (the whole plant treated), a positive correlation was observed by treated with vegetable oil (toxicity vs adaxial) at both concentrations (Supplementary Table 3).

Discussion

Mites dispersal did through walking (Sabelis & Dicke, 1985) to find a suitable site for colonization and feeding (Tixier et al., 2000; Aguilar-Fenollosa et al., 2016; Moerman, 2016; Mukwevho et al., 2017; Sousa et al., 2019). One major factor for dispersal is environmental contamination, as done by the pesticide application (Lima et al., 2015; Guedes et al., 2016; Mohammed et al., 2019; Monteiro et al., 2019b). This study aimed to determine whether synthetic chemicals and oils respond similarly to the dispersal and colonization behavior of *Panonychus citri*. The physio-morphic characteristics of leaf such as leaf surfaces (adaxial and abaxial) and leaf domatia play an essential role in habitat selection (O'Dowd & Pemberton, 1994, 1998; Tixier et al., 2000; English-Loeb et al., 2002; Romero & Benson, 2004). The majority of mites (Tetranychids) prefer to feed and oviposit on the leaves' abaxial surface. In contrast, some phytophagous mites like *P. citri* and *Tetranychus urticae* preferred on both surfaces (Azandeme-Hounmalon et al., 2014). This mites distribution from treated surfaces due to chemical cues (Domingos et al., 2010; Melo et al., 2011) and maybe their phylogenetical responses (Rollo et al., 1994; Nilsson & Bengtsson, 2004; Cisak et al., 2012; Buehlmann et al., 2014).

In the citrus growing region of South China, SYP-9625 and abamectin commonly used against different pests, including citrus red mite (Meng et al., 2002; Fang et al., 2013; Huixia et al., 2013; Liao et al., 2016; Dou et al., 2017). SYP-9625 is commonly used against phytophagous mites with minimum hazard to animals (Li et al., 2010; Chai et al., 2011; Huixia et al., 2013; Yu et al., 2016; Liu et al., 2018; Ouyang et al., 2018; Chen et al., 2019). Liu et al. (2018) reported that SYP-9625 gave maximum mortality and dispersment against *P. cirti* in the no-choice test, similar to our results and against *Tetranychus citri* (Chen et al., 2019). Abamectin showed less repellency than SYP-9625 against *P. citri* (Dou et al., 2017) due to resistance development (NATESC, 2003; Hu et al., 2010; Liao et al., 2016).

By contrast to synthetic chemicals, plant-based derivatives (Allelochemicals) used as alternatives (Flamini, 2003) due to compatible with non-target organisms, low toxic, negligible resistance development, and eco-friendly (Marcic, 2012). Fatty acids (saturated and unsaturated) are significant vegetable oils components as an active ingredient to increase its toxicity against pests (Baldwin et al., 2009; Sims et al., 2014). Linoleic acid resulted in attractive responses (Rollo et al., 1994; Buehlmann et al., 2014), as *P. citri* found on treated surfaces (at LC_{50}) after 24 hours in this study. The short-chain compound (palmitic acid) in vegetable oil gave equal repellency to synthetic chemicals (Mullens et al., 2009; Buehlmann et al., 2014). Vegetable oils gave similar responses to synthetic chemicals with a slow mode of action. They can be used as an alternative against *P. citri* with Ribeiro et al. (2014) endorsement.

EnSpray 99 exhibits minimum toxic residues on the treated fruit surfaces due to losing their toxicity (Zhuang et al., 2015). The efficacy of EnSpray 99 reported against different pests, including citrus red mites by many researchers (Wang et al., 2004; Chen & Zhan, 2007; Tao &

Xiao-fang, 2011; Teifemg et al., 2011; Zhuang et al., 2015). The EnSpray 99 contains paraffinic oil more than 60%, which was also found on the fruit residues (Ahmad et al., 2018) and effectively used for *P. citri* (Riehl & Jeppson, 1953; Trammel, 1965). The study shows that EnSpray 99 responded similarly to vegetable oil and synthetic chemicals against the repellency and dispersal of *P. citri*. The recommended concentrations ranging from 0.5 to 1.4% against *P. cirti* and eriophyids (Benfatto et al., 2002; Tang et al., 2002) while Wang et al. (2004) used 14.11 mgL^{-1} (LC_{50}) against *P. citri* in the laboratory. EnSpray 99 can be used against *P. citri* control strategies by keeping their impact on pest resistance development, environmental contamination, plant growth reduction, and chronic and acute effect on humans (Ahmed & Fakhruddin, 2018).

According to free-choice bioassay on dispersal, all treatments were significantly dispersed towards the un-treated and half-treated surfaces. According to Alves et al. (2005), untreated surfaces were significantly preferred by the *P. citri* at the adult stage for feeding and oviposition. Maximum dispersal from treated to un-treated or half-treated surfaces depended on the concentration of chemicals, which endorsed by Iftner & Hall (1983) by evaluating the pyrethroids against *T. urticae*. The dispersal towards half-treated adaxial surfaces was significantly different from vegetable oil application than others at $LC_{30,}$ as observed by Alves et al. (2018), treating the dimethoate against *P. citri*.

The no-choice (the whole plant treated) bioassay, selection of suitable surfaces by *P. citri* were observed non-significant differences between the number of mites captured on the abaxial and adaxial treated surfaces at LC_{50}. By evaluating the LC_{30} doses, significant differences were observed between the adaxial and abaxial surfaces in all treatments except control. Adaxial surfaces of leaflets were the most preferred site for feeding at LC_{30}. In no-choice (the whole pant treated) bioassay, oils give more repellency than synthetic chemicals.

The comprehensive assessments of these chemicals against *P. citri* need a more detailed study. The surface treated with these chemicals may affect natural enemies efficiency. However, the experiment carried out here did not evaluate the fundamental factors like environmental and needed attention to more applied work.

Conclusions

In conclusion, results show that: 1) *P. citri* dispersed away from treated surfaces; 2) among the leaf surfaces, the adaxial is a more preferred site for feeding and colonization; 3) a maximum number of mites missing or found dead by SYP-9625 application; 4) among the acaricides tested, oils were the least affecting the colonization depending on mite release point; 5) lethal doses allowed the minimum settlement of mites on the surface as compared to sublethal doses.

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Table 1 (on next page)

Toxicity of *Panonychus citri* (% mortality ± SE) 24 hours within nine experiment combinations.

Capital letters indicate the differences among the LC_{30} of treatments with control and lowercase indicates differences among the LC_{50} of treatments with a control. Different letters in the same column are significantly different at the Tukey test (α = 0.05).

Table 1: Toxicity of *Panonychus citri* (% mortality ± SE) 24 hours within nine experiment combinations. Capital letters indicate the differences among the LC_{30} of treatments with control and lowercase indicates differences among the LC_{50} of treatments with a control. Different letters in the same column are significantly different at the Tukey test (α = 0.05).

Treatment	Concentrations (%)	Experiments 1	2	3	4	5	6	7	8	
SYP-9625	LC_{30}	10 ± 0 A	0 ± 0 B	8.889 ± 1.11 A	5.556 ± 1.11 A	6.667 ± 0 A	5.556 ± 2.22 A	13.33 ± 1.925 A	6.667 ± 0 A	
	LC_{50}	12.223 ± 2.22 a	2.22 ± 1.11 b	12.22 ± 1.11 a	13.33 ± 1.925 a	8.889 ± 2.22 a	7.778 ± 1.11 a	17.778 ± 1.11 a	7.778 ± 1.11 a	
Abamectin	LC_{30}	2.22 ± 1.11 BC	2.22 ± 1.11 B	0 ± 0 B	2.22 ± 1.11 B	7.778 ± 1.11 A	3.33 ± 0 A	10 ± 0 A	3.33 ± 1.925 A	
	LC_{50}	6.667 ± 0 ab	3.33 ± 0 b	5.556 ± 1.111 b	6.667 ± 0 abc	12.22 ± 2.22 a	4.44 ± 1.11 ab	16.667 ± 1.925 a	4.44 ± 1.11 ab	
Vegetable oil	LC_{30}	7.778 ± 1.11 AB	0 ± 0 B	0 ± 0 B	0 ± 0 B	3.33 ± 0 A	2.22 ± 2.22 A	6.667 ± 1.925 AB	1.11 ± 1.11 A	
	LC_{50}	13.33 ± 1.925 a	2.22 ± 1.11 b	2.222 ± 1.111 bc	3.33 ± 1.925 bc	5.556 ± 1.11 ab	6.667 ± 0 a	12.22 ± 1.11 a	5.556 ± 1.11 ab	
EnSpray 99	LC_{30}	12.22 ± 1.11 A	7.778 ± 1.11 A	0 ± 0 B	0 ± 0 B	4.444 ± 1.11 A	0 ± 0 A	8.889 ± 2.22 A	4.44 ± 2.22 A	7. 1.
	LC_{50}	12.22 ± 2.94 a	8.889 ± 1.11 a	0 ± 0 c	8.889 ± 1.11 ab	7.778 ± 1.11 a	6.667 ± 1.925 a	13.33 ± 3.849 a	5.556 ± 1.11 ab	7. 1.
Control		0 ± 0 B, b	0 ± 0 B, b	0 ± 0 B, c	0 ± 0 B, c	0 ± 0 B, b	0 ± 0 A, b	0 ± 0 B, b	0 ± 0 A, b	0
Statistics at $df = 4,14$	LC_{30}	$F = 18.08$ $P = 0.000$	$F = 23$ $P = 0.000$	$F = 64$ $P = 0.000$	$F = 12$ $P = 0.001$	$F = 12.17$ $P = 0.001$	$F = 2.05$ $P = 0.164$	$F = 7.69$ $P = 0.004$	$\boldsymbol{F = 3.33}$ $\boldsymbol{P = 0.056}$	*F* *P*
	LC_{50}	$F = 7.50$ $P = 0.005$	$F = 7.50$ $P = 0.005$	$F = 35.50$ $P = 0.000$	$F = 10.60$ $P = 0.001$	$F = 8.35$ $P = 0.003$	$F = 4.88$ $P = 0.019$	$F = 11.97$ $P = 0.001$	$F = 5.83$ $P = 0.011$	*F* *P*

Figure 1

Figure 1. Systematic outline of the experimental layout.

(A) Mites were released on the right adaxial (ADR) surfaces; B) ADR and ADL; C) ABR and ABL; D) ADR and ABR; E) ADL and ABL; F) ADR and ABL; G) ABR and ADL; H) for full treated ADR and ABR, and for half treated, ADL and ABL; I) for full treated ADL and ABL, and for half treated ADR and ABR, and J) whole plant treated.

Letters were assigned to leaves surfaces as; adaxial right (ADR), adaxial left (ADL), abaxial right (ABR), and abaxial left (ABL).

Photo credit: Muhammad Asif Qayyoum.

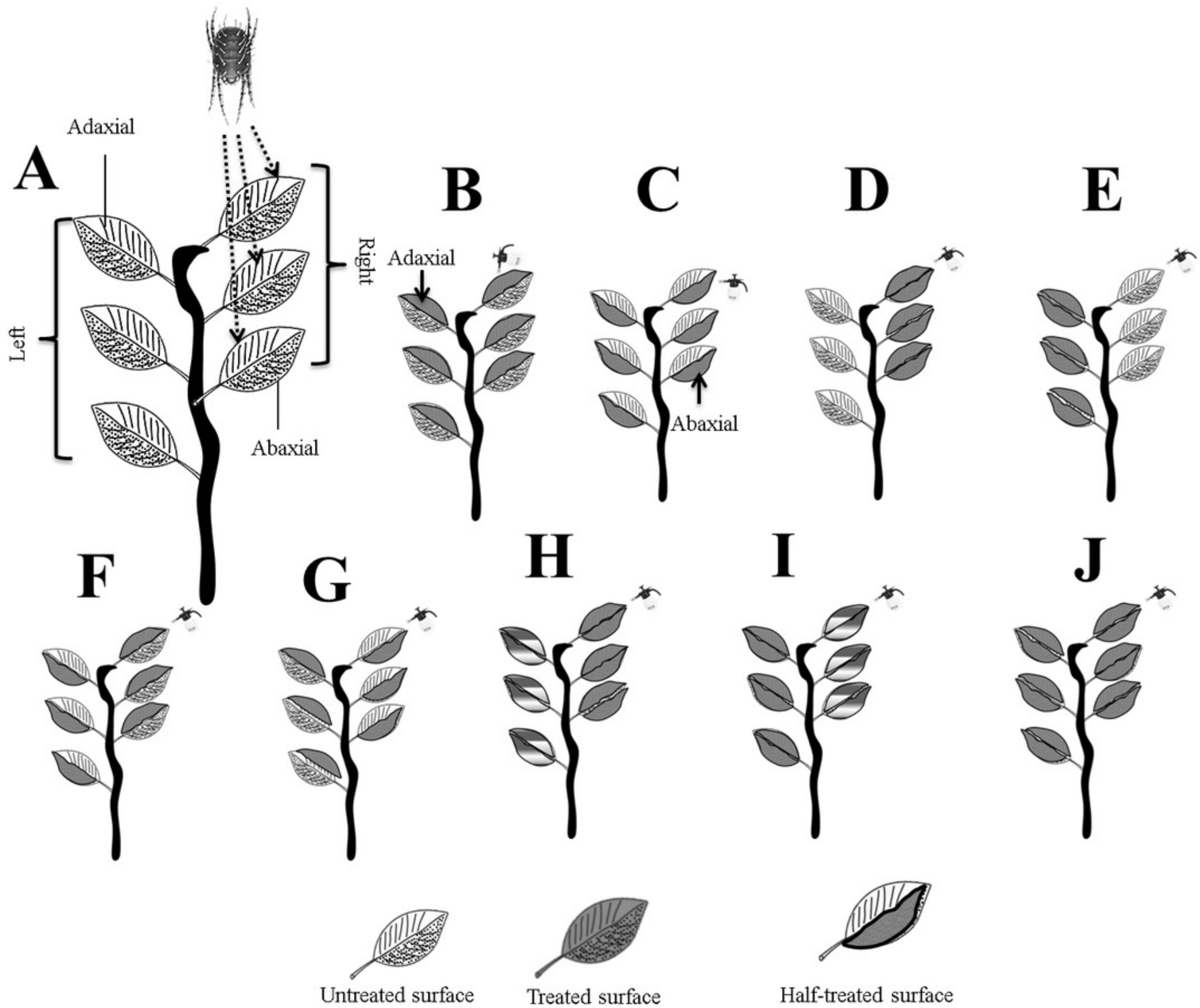
A
Adaxial
Left
Right
Abaxial
B
Adaxial
C
Abaxial
D
E
F
G
H
I
J
Untreated surface
Treated surface
Half-treated surface

Figure 2

The number of *Panonychus citri* (Mean ± SE) re-captured after 24 hours on the adaxial surface of leaves (right side); (A) LC_{30}, (B) LC_{50}.

A significant difference was observed between treatments than control within treated (*df* = 4,14; For LC_{30}: *F* = 3.37, *P* = 0.018; for LC_{50}: *F* = 28.01, *P* = 0.000) and untreated surfaces (For LC_{30}: *F* = 7.41, *P* = 0.000; for LC_{50}: *F* = 4.74, *P* = 0.003). The capital letters indicate differences among the treatments (Treated or Un-Treated); lowercase indicates differences between treated and untreated surfaces, Tukey test (α = 0.05). Significant difference "***" and non-significant difference "ns".

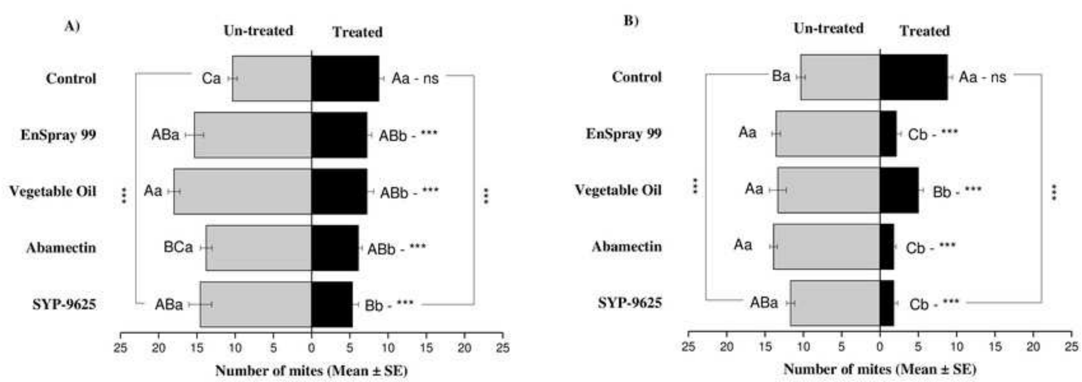

Figure 3

The number of *Panonychus citri* (Mean ± SE) re-captured after 24 hours on the adaxial surface of leaves (left side); (A) LC_{30}, (B) LC_{50}.

A significant difference was observed between treatments than control within treated at LC_{50} ($F = 14.67$, $P = 0.000$). The capital letters indicate differences among the treatments (Treated or Un-Treated); lowercase indicates differences between treated and untreated surfaces, Tukey test ($\alpha = 0.05$). Significant difference "***" and non-significant difference "ns".

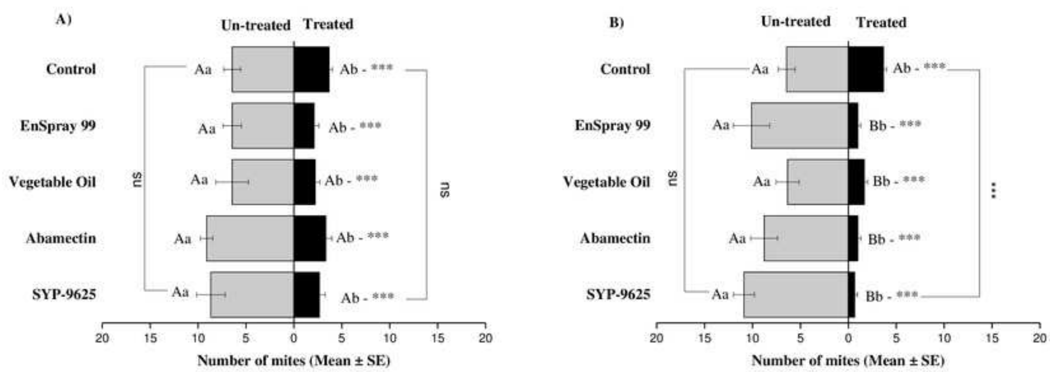

Figure 4

The number of *Panonychus citri* (Mean ± SE) re-captured after 24 hours on the abaxial surface of leaves (right side); (A) LC_{30}, (B) LC_{50}.

A significant difference was observed between treatments than control at LC_{50} (For treated: $F = 10.86$, $P = 0.000$; for untreated: $F = 4.89$, $P = 0.003$). The capital letters indicate differences among the treatments (Treated or Un-Treated); lowercase indicates differences between treated and untreated surfaces, Tukey test ($\alpha = 0.05$). Significant difference "***" and non-significant difference "ns".

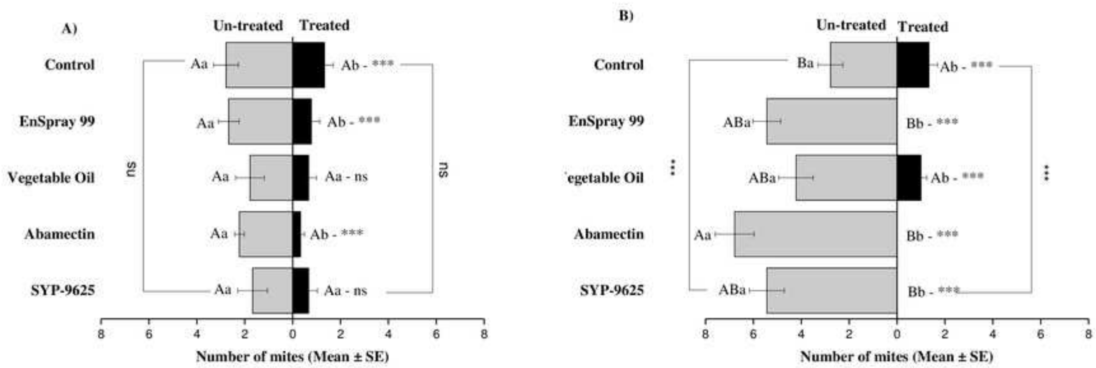

Figure 5

The number of *Panonychus citri* (Mean ± SE) re-captured after 24 hours on the abaxial surface of leaves (left side); (A) LC_{30}, (B) LC_{50}.

A significant difference was observed between treatments than control within treated surfaces (df = 4,14; For LC_{30}: F = 15.01, P = 0.000; for LC_{50}: F = 29.78, P = 0.000). The capital letters indicate differences among the treatments (Treated or Un-Treated); lowercase indicates differences between treated and untreated surfaces, Tukey test (α = 0.05). Significant difference "***" and non-significant difference "ns".

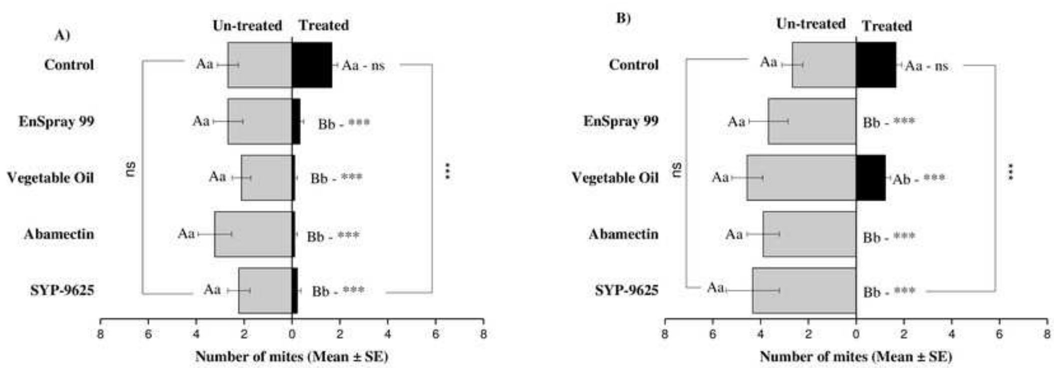

Figure 6

The number of *Panonychus citri* (Mean ± SE) re-captured after 24 hours on the adaxial surface of leaves; (A) LC_{30}, (B) LC_{50}.

A significant difference was observed between treatments than control within treated (df = 4,29; at LC_{50}: F = 8.55, P = 0.000). The capital letters indicate differences among the treatments (Treated or Half-Treated); lowercase indicates differences between treated and half-treated surfaces, Tukey test (α = 0.05). Significant difference "***" and non-significant difference "ns".

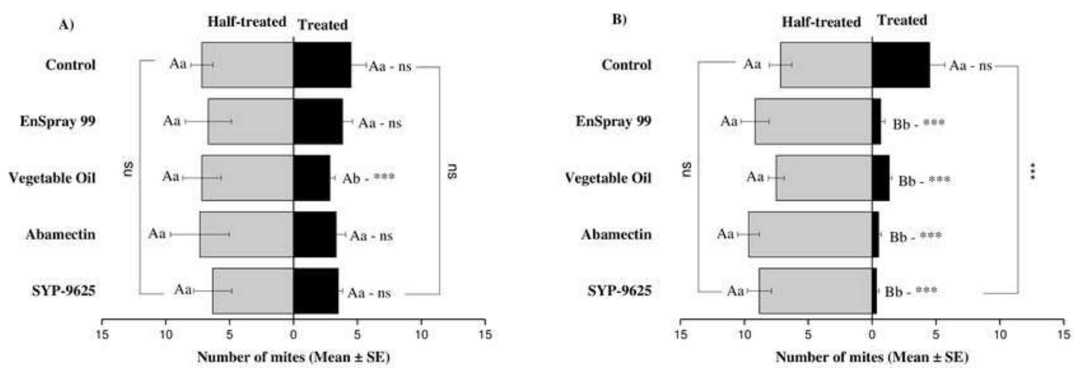

Figure 7

The number of *Panonychus citri* (Mean ± SE) re-captured after 24 hours on the abaxial surface of leaves; (A) LC_{30}, (B) LC_{50}.

A significant difference was observed between treatments than control within treated (*df* = 4,29; at LC_{50}: $F = 29.61$, $P = 0.000$). The capital letters indicate differences among the treatments (Treated or Half-Treated); lowercase indicates differences between treated and half-treated surfaces, Tukey test (α = 0.05). Significant difference "***" and non-significant difference "ns".

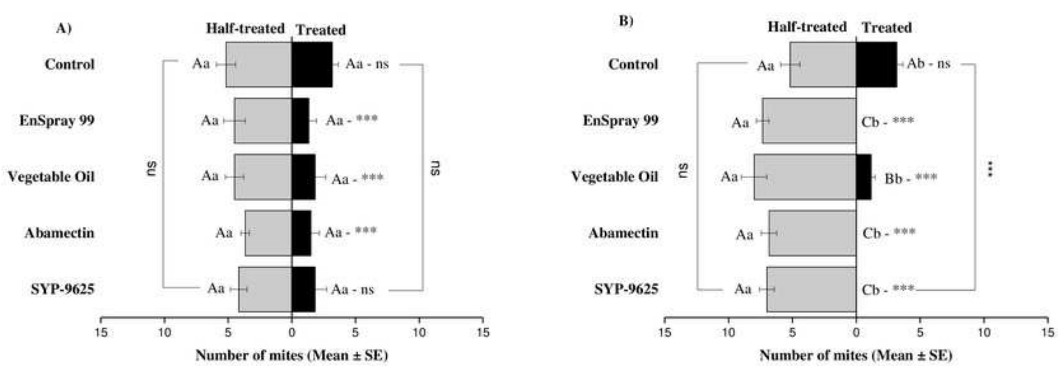

Figure 8

The number of *Panonychus citri* (Mean ± SE) re-captured after 24 hours (the whole plant treated) on adaxial and abaxial surfaces. The results of LC30 (A) and LC50 (B) concentrations are presented.

A significant difference was observed between treatments than control within abaxial (df = 4,29; at LC_{30}: F = 8.32, P = 0.000). The capital letters indicate differences among the treatments (Adaxial or Abaxial surface); lowercase indicates differences between adaxial and abaxial surfaces, Tukey test (α = 0.05). Significant difference "***" and non-significant difference "ns".