

Full Length Research Paper

Evaluation of neem leaves-based preparations as insecticidal agents against the green peach aphid, *Myzus persicae* (Sternorrhyncha: Aphididae)

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Myzus persicae Sulzer, 1776 (Sternorrhyncha: Aphididae) is an insect pest of several crops. Chemical control against pests causing harmful effects, so it is necessary to find alternative methods. In this context, insecticidal activity of water and hydroethanol extracts of neem leaves was investigated against the aphid *M. persicae* through biological performance and feeding behavior assessment. Water and hydroethanol extracts of neem leaves are prepared by soaking ground leaves in water or 10% ethanol overnight. Both water and hydroethanol extracts of neem leaves at 1, 5 and 10% in artificial diet significantly reduced ($p < 0.05$) the survival of nymphs, leading to more than 95% mortality. Both extracts at 0.1% significantly reduced ($p < 0.05$) the survival rate and fecundity of *M. persicae* adults. The feeding behavior of aphids was studied with a dual-choice assay and using the electrical penetration graph (EPG) technique. Dual-choice assays revealed the aphid rejection of the water extract at 10% and the hydroethanol extract at 1 and 5% concentrations. EPG monitoring showed enhanced duration of probing and ingestion on artificial diet containing the water extract at 10%. On plants sprayed with water extract at 10%, EPG monitoring showed reduced duration of probing, delayed phloem access, and reduced salivation and ingestion phases. Our results showed that neem leaves-based preparations are insecticidal agents and effective to control *M. persicae*.

Key words: Neem leaves extracts, *Myzus persicae*, survival, feeding behavior.

INTRODUCTION

Crop damages caused by aphids (Sternorrhyncha: Aphididae) is one of the most serious problems in

agriculture. These sap suckers may directly alter plant metabolism through removal of phloem sap and injection

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of salivary secretions inducing morphological changes, sap modification and various local as well as systemic symptoms (Giordanengo et al., 2010) like discoloration of organs, necrosis and windings of leaves. However, the most important damages caused by aphids are indirect. They stem from many phytopathogens transmitted to plants causing severe yield losses (van Emden and Harrington, 2007). Distributed worldwide, the peach potato aphid *Myzus persicae* (Sulzer) is a highly polyphagous species capable of infecting plants in more than 40 different plant families including many economically important plants like peach, potato and cabbage on which it can vector up to 100 phytoviruses (van Emden and Harrington, 2007).

To date, control of aphids mainly relies on the use of pesticides but the rise of environmental concerns (Devine and Furlong, 2007) and the emergence of insecticide resistance risk (Foster et al., 1998; Anstead et al., 2005) have led to search of alternative strategies to control population outbreaks.

Insecticidal properties of neem (*Azadirachta indica* A. Juss, Meliaceae) are traditionally used in cultural practices from several thousand years (Philogène et al., 2003). Neem compounds present various effects ranging from repellency to toxicity against a wide spectrum of insect pests including Orthoptera, Lepidoptera, Coleoptera, Diptera and Hemiptera (Schmutterer, 1990; Isman, 2006; Siddiqui et al., 2009; Degri et al., 2013; Shannag et al., 2014). These biological properties are mediated by different groups of compounds among which limonoids and particularly azadirachtin mainly present in the neem seeds are considered the most active components responsible of both antifeedant and insecticidal effects (Isman, 2006). Neem-based insecticides have low environmental impact because of a rapid degradation in plants and in the soil (Isman, 2006) and low effects on beneficial insects (Tang et al., 2002; Haseeb et al., 2004; Defago et al., 2011). Moreover, azadirachtin has been proved non-toxic to vertebrates (Mordue, 2004; Isman, 2006) and therefore neem extracts represent a valuable tool to control population outbreaks in integrated pest management programs.

The problem is that several commercial formulations containing azadirachtin are available on the world market for insect control (Boursier et al., 2011) but refined products are too costly for developing countries (Isman, 2006; Boursier et al., 2011). Aqueous extract of seeds is traditionally used in malian cotton fields to fight Hemiptera pests and the pathogens they vectored (Boursier et al., 2011). Despite two fructification periods per year, their discontinuous availability limits the use of seed-based preparations. Interestingly, numerous active compounds including limonoids have also been found in neem leaves (Siddiqui et al., 2000; Afshan, 2002) which extracts were shown to exert insecticidal effects (Brunherotto et al., 2010; Egwurube et al., 2010).

Insecticidal activity of water and hydroethanol extracts prepared from roughly ground neem leaves has been

confirmed to protect cabbage against Lepidoptera (Akantétou, 1990) and Sternorrhynca pests in fields in Togo (Mondédji, 2010). Owing to such potential of neem leaves-based preparations to control insect populations, our hypothesis is that these preparations affect *M. persicae* survival (antibiosis) after ingestion and/or are antifeedant for it by changing its feeding behavior (antixenosis). We have focused our study on two main objectives: 1) to evaluate aphicidal (antibiosis) efficiency of neem leaves extracts and 2) identify their nature (antixenosis) to assess the process of preparing neem leaves extracts to enable producers to produce by themselves and throughout the year.

MATERIALS AND METHODS

Plants and insects

Potato plants, *Solanum tuberosum* L. (Solanaceae) cv. Désirée, were grown from tubers in 9 cm plastic pots filled with peat moss-based potting medium in a growth room maintained at $20 \pm 1^\circ\text{C}$, $65 \pm 5\%$ H.R. and L16:D8 photoperiod. The peach potato aphid, *M. persicae* Sulzer, colony was started from a single virginiparous female collected in early summer 1999, from a potato field near Loos-en-Gohelle, France ($50^\circ27'27''\text{N}$, $2^\circ47'30''\text{E}$). Aphids were reared in a separate growth room on potted *S. tuberosum* plants at $20 \pm 1^\circ\text{C}$, $65 \pm 5\%$ H.R. and L16:D8 photoperiod.

Elaboration of leaves extracts and diets

Neem, *A. indica* A. Juss. (Meliaceae), leaves were collected on neem trees in the Lomé University campus. Extracts were obtained by soaking 1 kg of grinded fresh leaves in 1.5 L water or 10% hydroethanol solution overnight at 25 to 30°C . The preparations were then filtrated through a Whatman No 1 filter paper to obtain the crude neem leaves water or hydroethanol extract (hereafter referred as W or H, respectively).

An artificial diet adapted for *M. persicae* was used as a carrier for neem extracts dilution and as a negative control (C). Artificial diet added with 10% ultrapure water (CW) or 10% hydroethanol solution at 10% (v/v with ultrapure water) (CH) was used as positive control. The diet was prepared as described by Febvay et al. (1988) and modified by Down et al. (1996). W or H was incorporated to the artificial diet to obtain diets W0.1, H0.1, W1, H1, W5, H5, W10 and H10, containing respectively 0.1, 1, 5 and 10% (v/v) of W or H. After preparation, the diets were passed through a $0.2 \mu\text{m}$ filter (Millipore Corp., Bedford, Massachusetts, USA). Parafilm® pouches ($80 \mu\text{l}$) were prepared under aseptic conditions.

Effects of neem leaves extracts on aphid demographic parameters

Five nymphs younger than 24 h were transferred to a new pouch of each diet (C, CW, CH, W0.1, H0.1, W1, H1, W5, H5, W10 and H10) and maintained under the same rearing conditions as described in the section "Plants and insects". Diet pouches were changed every second day. Ten replicates were carried out for each diet. Nymphal survival, prereproductive period (that is, the period of time from birth until onset of reproduction), adult emergence Le Roux et al. (2004), reproduction and survival were recorded every 2 days according to the Jackknife method (Meyer et al., 1986) was used to evaluate the variance of the intrinsic rate of natural increase (r_m , $\sum e^{-r_m x} . l_x . m_x = 1$)

with the DEMP 1.5 software (Giordanengo, 2010).

The effects of neem leaves extracts on nymphal survival were analysed with Pearson's χ^2 test. One-way analysis of variance (ANOVA) was carried out to test the effects of the diets on aphid demographic parameters. Significantly altered demographic parameters were further analyzed with Fisher's positive least significant difference test (PLSD). To determine the LD₅₀, dose-mortality relationship was determined using WIN DL (CIRAD-CA/MABIS, Montpellier, France), based on probit analysis (Finney, 1971).

Effects of neem leaves extracts on aphid feeding behavior

Dual-choice assay

The dual-choice assay was realized using the device described by Sauvion et al. (2004). It consisted of Plexiglas® built-in cylindrical boxes (7.2 mm diameter, 6 mm high) closed by two pouches of diet (20 μ l) diametrically opposite. One wingless adult aphid aged less than 24 h was inserted in the device and aphid position relative to the pouches was recorded after 6 h. The experiment was conducted in the dark in a growth room maintained at $20 \pm 1^\circ\text{C}$. The diets (that is, CW, CH, W0.1, H0.1, W1, H1, W5, H5, W10 or H10) and the control (C) were alternated and the device cleaned with TFD4 detergent (Franklab, St-Quentin en Yvelines, France) between two replications. Thirty six replicates were done for each treatment. For each experiment, the distribution of responding aphids was analyzed using a Wilcoxon test (Z) for paired samples. We used a Kruskal-Wallis test (H) to compare the percentages of non-responding aphids between experiments.

Stylet activities

Stylet penetration activities during aphid feeding behaviour were studied using the DC electrical penetration graph (EPG) (Tjallingii, 1988). A 2 cm gold wire (20 μ m in diameter) was pasted on the aphid's dorsum by conductive silver glue. For *in planta* experiments, three-weeks old potted plants were treated using a hand-held trigger spray (Pulsar 1 L, Tecnomat, Epernay, France) to apply 6 ml of W (PW), 10% W diluted with distilled water (v/v) (PW10) or distilled water as control (PC) to each plant. A treated plant (PW, PW10 or PC) was connected to the system via a copper electrode stuck in the potting medium. Each connected aphid was carefully placed on the abaxial surface of the fourth fully expanded leaf of the potato plant and monitored for 8 h. For *in vitro* experiments, the second electrode was inserted through the Parafilm® into a pouch filled with W10 or standard diet as control. Aphids were monitored for 4 h.

The feeding behavior of eight wingless adult aphids aged less than 24 h, each feeding on a separate plant or pouch, were monitored simultaneously using a Giga 8™ amplifier with $10^9 \Omega$ input resistance (EPG-systems, Wageningen, The Netherlands) in an electrically grounded Faraday cage to shield the setup from external electrical noise. All experiments were carried out in a growth room maintained at $20 \pm 1^\circ\text{C}$. At least 18 aphids were monitored for each treatment. The recordings consistently started around 10:00 h (± 30 min). Data acquisition and analyses were done with PROBE 3.5 software (EPG-systems, Wageningen, The Netherlands), according to Tjallingii and Hogen Esch (1993). Eighteen to 21 replications were performed for each series. Waveforms A, B, C, and pd (potential drop, that is, brief intracellular puncture) were grouped and labelled as 'C pattern' corresponding waveforms, E1 (salivation into the sieve elements), E2 (passive to the advancement of the stylets in the plant tissues). Other ingestion of phloem sap), G (active ingestion of xylem sap), and F (derailed

stylet mechanics) were also identified. Electropenetrography parameters were calculated using EPG-CALC 4.9 software (Giordanengo, 2014).

Fifteen parameters organized into five classes (Table 3) were chosen to describe the effects of plant treatments on the probing behavior of *M. persicae*. The 'general probing behavior' class included the mean number of probes, the total duration of probing and the time from the beginning of the monitoring to the first stylet insertion. The 'pathway phase' class corresponded to probing activities in non-vascular tissues and included the mean number and the total duration of pathway phases. The 'phloem salivation' class included the number and the total duration of salivation bouts with a distinction between single phloem salivation periods and phloem salivation periods followed by sap ingestion, and duration from the first probe before the first salivation. The 'phloem ingestion phase' class included the mean number and the total duration of phloem sap ingestion bouts, and the duration from the first probe to the first ingestion. Fifth class, 'other parameters' included the total duration of xylem ingestion and stylets derailment bouts.

For *in vitro* experiments, only C, E1 and G waveforms were recorded. The latter corresponding to the active ingestion of the artificial diet (Sauvion and Rahbé, 1999) consistently differed from that observed for aphids feeding on plants since passive phloem sap ingestion (E2) is forced by the pressure in the sieve tubes (Miles, 1999; Tjallingii and Cherqui, 1999). To describe the effects of W10 diet on the probing behaviour of *M. persicae*, we selected 9 parameters organized into three classes (Table 4). The 'general probing behavior' class included the mean number of probes, the total duration of probing and the time from the beginning of the monitoring to the first stylet insertion. The 'salivation phase' class included the number and the total duration of salivation bouts and the total duration from the first probe before the first salivation. The 'ingestion phase' class included the mean number and the total duration of diet ingestion bouts, and the duration from the first probe to the first ingestion. Pair-wise comparison between each treatment and its respective control was performed with a Mann-Whitney U-test to analyze the effects of plant treatment or diet on the EPG data. All statistical analyses were performed using STATISTICA 6 software (StatSoft, Tulsa, OK, USA).

RESULTS

Effects of neem leaves extracts on aphid demographic parameters

Nymph survival (Figure 1) was not affected by adding 10% water (CW: $p > 0.05$) or 10% hydroethanol solution (CH: $p > 0.05$) (positive controls) in the artificial diet, when compared with the artificial diet alone (C) (negative control). Whatever the dose, both water (W0.1: $p < 0.002$; W1: $p < 0.001$; W5: $p < 0.001$; W10: 3, $p < 0.001$) and hydroethanol (H0.1: $p < 0.01$; H1: $p < 0.001$; H5: $p < 0.001$; H10: $p < 0.001$) extracts added to the diet significantly increased *M. persicae* nymph mortality. LD₅₀ as a percentage of crude hydroethanol or water extract was determined at 0.37 and 0.43% (Table 1) with equations of mortality linear regression $Y = 0.41 + 0.95X$ and $Y = 0.33 + 0.92X$ respectively.

Demographic parameters were only calculated for aphids reared on W0.1 and H0.1 diets on which adults were obtained (Table 2). Significantly extended prereproductive period and reduced daily fecundity and

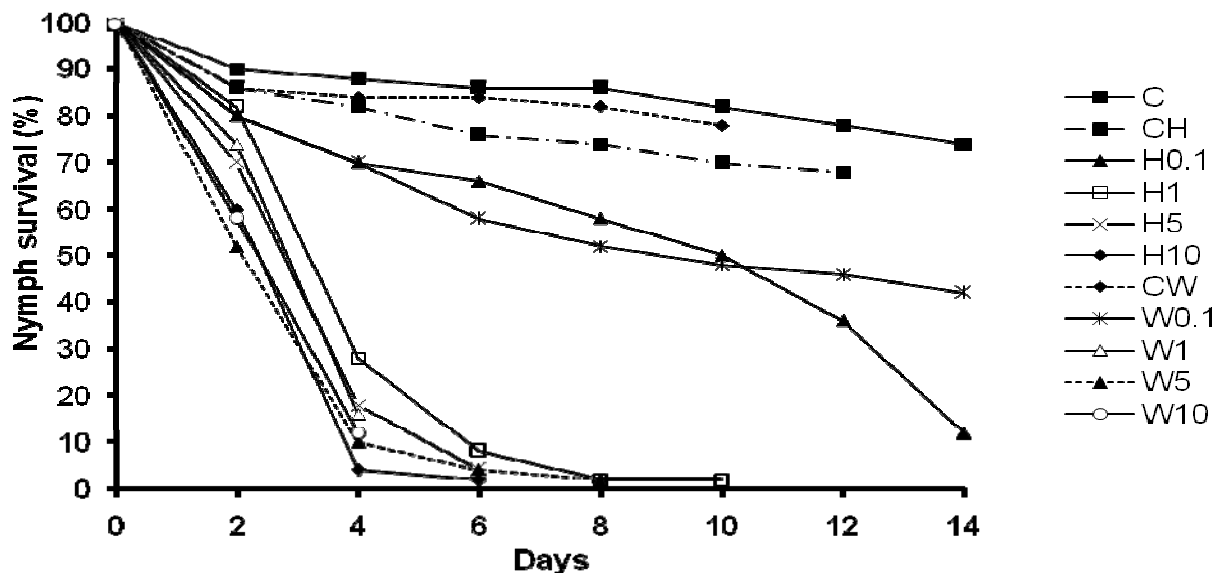


Figure 1. Survival of *Myzus persicae* nymph reared on artificial diet (C) as negative control or artificial diet added with hydroethanol (H) or water (W) neem leaves extract at 0.1 (H0.1 and W0.1), 1 (H1 and W1), 5 (H5 and W5) or 10 (H10 and W10)% (v / v).

Table 1. Dose-mortality relationship with LD50 with inferior and superior limits of the 95% confidence interval for water and hydroethanol extracts of neem leaves delivered via ingestion route to *Myzus persicae*.

Extracts	Inf lim < LD50* < sup lim
Water	0.011 < 0.434 < 1.085
Hydroethanol	0.008 < 0.371 < 0.961

*LD50 is expressed as a ratio of crude neem leaves water or hydroethanol extract diluted artificial diet (v / v).

oviposition period were observed for adults whatever water or hydroethanol extracts added to the diet. Intrinsic rate of natural increase (r_m) and finite ratio of increase (λ) were therefore significantly reduced.

Effects of neem leaves extracts on aphid feeding behavior

Dual-choice assay

A blank test (control vs. control, $p > 0.05$) confirmed the absence of any bias in the dual-choice setup. Whatever water or hydroethanol dose tested, the percentage of non-responding aphids was not statistically different between experiments ($p > 0.05$). No positive control (CW, $p > 0.05$ and CH, $p > 0.05$) influenced aphid distribution (Figure 2). Neither 0.1, 1 nor 5% concentration of neem water extract in the artificial diet led to a non randomly distribution (W0.1, $p > 0.05$; W1, $p > 0.05$; W5, $p > 0.05$). Rejection of neem water extract occurred at 10%

concentration (W10, $p < 0.001$). No preference for the 0.1 or 10% neem hydroethanol extract added diets was shown (H0.1, $p > 0.05$; H10, $p > 0.05$). *M. persicae* significantly avoided the pouches containing 1 and 5% of neem hydroethanol extracts (H1, $p < 0.05$; H5, $p < 0.05$).

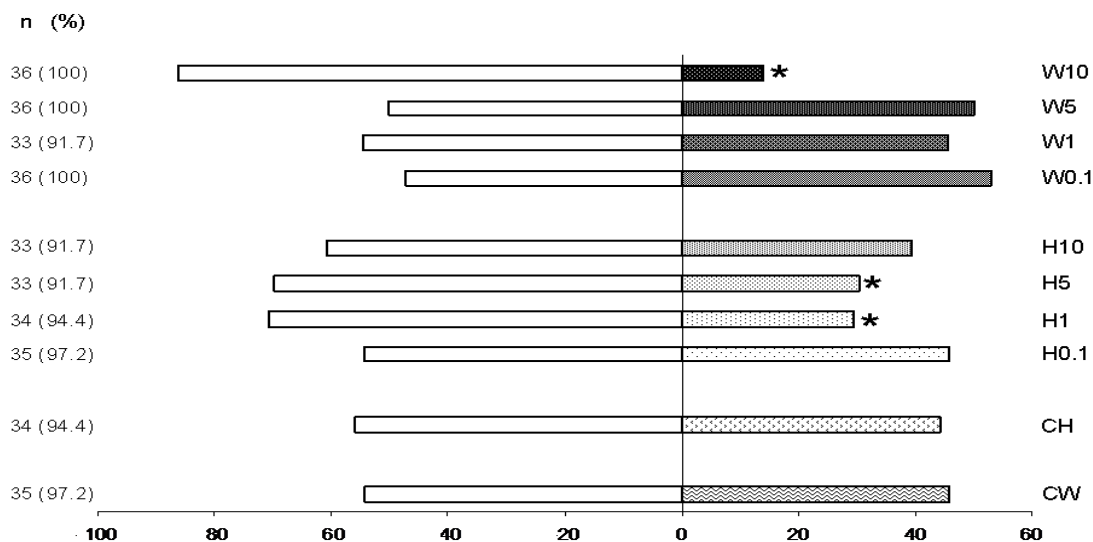
Stylet activities

In vitro monitoring: General probing behavior of *M. persicae* was affected on diet added with 10% neem water extract (W10 diet), aphids showing significantly enhanced duration of probing (parameter 2) (Table 3). None of the parameters describing the salivation phase was significantly affected. Ingestion phases were altered on W10 diet as aphids spent more time ingesting diet (parameter 8).

In planta monitoring: On W10 sprayed potato plants (PW10) the total duration of probing, the number of single salivation phases and the total duration of both single and

Table 2. Average (\pm SE) of demographic parameters for *Myzus persicae* reared on control diet (C) or on diet containing 0.1% (v / v) neem leaves water (W0.1) or hydroethanol (H0.1) extract.

Demographic parameter	C n=39	W0.1 n=19	H0.1 n=14	F	P
Pre-reproductive period (days)	10.3 \pm 0.1 ^a	17.4 \pm 1.0 ^b	14.3 \pm 0.7 ^c	43.38	< 0.001
Daily fecundity (nymphs per female per day)	0.34 \pm 0.03 ^a	0.06 \pm 0.02 ^b	0.187 \pm 0.76 ^b	11.90	< 0.001
Oviposition period (days)	7.53 \pm 0.50 ^a	0.86 \pm 0.3 ^b	1.9 \pm 0.8 ^b	49.32	< 0.001
Adult survival (days)	11.3 \pm 0.8 ^a	8.8 \pm 0.8 ^b	6.3 \pm 1.0 ^b	7.83	< 0.001
r_m (females per female per day)	0.088 \pm 0.006 ^a	-0.064 \pm 0.024 ^b	0.042 \pm 0.003 ^c	28.37	< 0.001

**Figure 2.** Distribution of *M. persicae* adults in the dual-choice setup after 6h experiment C: negative control (artificial diet); CW: artificial diet added with 10% ultrapure water (positive control); CH: artificial diet added with 10% hydroethanol solution (positive control); H0.1, H1, H5, H10: artificial diet added with hydroethanol neem leaves extract at 0.1, 1, 5 or 10%; W0.1, W1, W5, W10: artificial diet added with water neem leaves extract (v / v).

fraction salivation bouts were reduced (Table 4). When monitored on those plants, *M. persicae* showed a delayed first salivation bout. All the parameters linked to phloem ingestion were

modified: the number and the duration of phloem ingestion phases were reduced and phloem ingestion delayed. Stylets derailment and xylem ingestion were not affected.

DISCUSSION

Both water and hydroethanol extracts intoxication through ingestion route showed dose-dependent

Table 3. Feeding behavior of *Myzus persicae* during 4 h access to standard diet (C) or standard diet added with 10% (v / v) neem leaves water extract (W10).

	C	W10 diet
EPG classes and related parameters	n=18	n=21
General probing behavior		
1. Number of probes	16.8 ± 2.9	22.1 ± 2.5
2. Total duration of probing	137.5 ± 19.0	332.6 ± 33.6*
3. Time from start of recording to first probe	8.0 ± 4.5	7.5 ± 3.1
Salivation phase		
4. Number of salivation phases	1.9 ± 0.4	1.9 ± 0.5
5. Total duration of salivation phases	11.0 ± 8.6	13.1 ± 7.0
6. Time from first probe to first salivation	36.6 ± 11.6	40.1 ± 9.3
Ingestion phase		
7. Number of ingestion phases	11.6 ± 1.9	15.6 ± 2.0
8. Total duration of ingestion phases	112.3 ± 14.4	239.1 ± 33.5*
9. Time from first probe to first ingestion	33.3 ± 14.4	18.9 ± 18.0

Means (± SE) followed by * are significantly different from control plants (Mann-Whitney *U*-test: $P < 0.05$). n, number of aphids; times and durations are expressed in min.

Table 4. Feeding behavior of *Myzus persicae* during 8 h access to leaves of *Solanum tuberosum* cv Désirée sprayed by 10% (v / v) neem leaves water extract diluted in ultrapure water (PW10) or ultrapure water as control (PC).

	Control (PC)	PW10
EPG classes and related parameters	n=22	n=21
General probing behavior		
1. Number of probes	46.9 ± 4.5	40.6 ± 7.8
2. Total duration of probing	303.4 ± 19.1	214.8 ± 23.8*
3. Time from start of recording to first probe	12.0 ± 2.7	40.6 ± 13.7
Pathway phase		
4. Number of pathway phases	52.8 ± 4.7	43.3 ± 6.0
5. Total duration of pathway phases	188.2 ± 13.3	167.8 ± 21.0
Phloem salivation phase		
6. Number of single salivation periods	3.9 ± 0.6	1.2 ± 0.4*
7. Total duration of single salivation phases	18.2 ± 3.5	9.0 ± 3.8*
8. Number of fraction salivation phases	2.1 ± 0.5	1.0 ± 0.4*
9. Total duration of fraction salivation phases	11.0 ± 2.5	3.5 ± 0.6*
10. Time from first probe to first salivation	128.3 ± 29.1	269.9 ± 36.8*
Phloem ingestion phase		
11. Number of phloem ingestion	1.9 ± 0.4	0.8 ± 0.3*
12. Total duration of phloem ingestion	30.9 ± 11.5	4.6 ± 1.6*
13. Time from first probe to first phloem ingestion	228.9 ± 37.9	342.9 ± 31.8*
Others parameters		
14. Total duration of stylet derailment	13.7 ± 6.1	2.2 ± 0.4
15. Total duration of xylem ingestion	44.7 ± 11.4	32.3 ± 5.4

Means (± SE) followed by * are significantly different from control plants (Mann-Whitney *U*-test: $P < 0.05$). n, number of aphids; times and durations are expressed in min.

effects of neem leaves extracts leading to more than 95% *M. persicae* nymphs mortality for 1, 5 and 10% doses. A drastic reduction of nymph survival caused by

azadirachtin or neem seed extracts was reported on several aphids species (Lowery and Isman, 1996; Tuncer and Aliniyee, 1998; Tang et al., 2002; Pavela et al.,

2004) among which *M. persicae* (Lowery and Isman, 1996). Such mortality of immature stages may result, at least partly, from the molting inhibition property of azadirachtin (Mordue and Blackwell, 1993; Tang et al., 2002; Isman, 2006) as surviving *M. persicae* nymphs grown on diet added with neem leaves extracts were smaller and most of them did not develop into adult. Amtul (2014) reported *A. indica* derived compounds as inhibitors of digestive alpha-amylase in insect pest *Tribolium castaneum*. When *M. persicae* nymphs developed into adults (that is, only for 0.1% neem leaves water or hydroethanol extracts treatments), enhanced pre-reproductive period was observed as reported on the filbert aphid *Myzocallis coryli* (Tuncer and Aliniyee, 1998) and the brown citrus aphid *Toxoptera citricida* (Tang et al., 2002) intoxicated with neem seed extracts.

Adult intoxicated with 0.1% neem leaves water or hydroethanol extracts exhibited drastically reduced fecundity (that is, both offspring per aphid per day and oviposition period) and survival. Alteration or even failure of offspring production of aphids exposed to neem seed oil or azadirachtin (Nisbet et al., 1994; Lowery and Isman, 1996; Tuncer and Aliniyee, 1998; Tang et al., 2002; Pavela et al., 2004) was ascribed to embryos mortality just before parturition (Nisbet et al., 1994; Lowery and Isman, 1996). For the 0.1% water extract (W0.1) the negative intrinsic rate of population increase (r_m) would result in population extinction.

Besides these antibiosis effects, dual-choice assays showed aphids rejection of 10% concentration of neem water extract, and 1 and 5% concentration of neem hydroethanol extracts. Drastic antifeedant effects of neem-treated leaves were reported on various grazing insects such as Lepidoptera (Blaney et al., 1990; Simmonds et al., 1990; Ma et al., 2000), Coleoptera (Streets, 1976), Orthoptera (Mordue et al., 1998) and on aphids (Koul, 1999). Aqueous plant extracts of *A. indica* showed feeding deterrence on the cabbage butterfly *Pieris brassicae* (Sharma and Gupta, 2009) and the diamondback moth *Plutella xylostella* (Charleston et al., 2005), and Singh et al. (1987) reported such antifeedant effect in leaf water extracts on *P. brassicae*. *In vitro* EPG monitoring showed enhanced duration of probing and ingestion when aphids fed on artificial diet added with 10% water extract. Ingestion of neem-seed oil does not immediately inhibit feeding (Morgan, 2009). As enhanced duration of ingestion phase monitored by EPG can not be directly linked with an increased amount of ingested diet it could be the consequence of difficulties to ingest as seemed to indicate enhanced probing duration. Such behavior recorded during 4 h would thus lead to reject the intoxicated diet as observed after 6 h in the dual-choice assays. This previous hypothesis is supported by *in planta* EPG monitoring which showed reduced duration of probing and delayed phloem phase (salivation and ingestion phases), and reduced salivation and ingestion phases indicating repellent properties perceived within

the plant tissues and antifeedant properties perceived in the phloem sap, respectively. Azadirachtin has been shown to act systemically (Isman, 2006; Morgan, 2009), however, taken together *in vitro* and *in planta* monitoring also suggest a deterrent effect perceived on the plant surface by chemosensory sensillae on the proboscis as reduced probing duration was only observed on sprayed plants. Such deterrence of surface treatment to aphids appears highly variable depending on the targeted species (West and Mordue, 1992; Lowery and Isman, 1993; Koul et al., 1997; Koul, 1999) and the nature of the sprayed extract (Koul, 1999).

Though neem leaves are reported a very poor source of azadirachtin (Morgan, 2009) numerous active compounds including limonoids have been identified in neem leaves (Siddiqui et al., 2000; Afshan, 2002). The composition of the extracts we dealt with was not investigated but whatever their water or hydroethanol nature, their effects on aphids survival and their LD₅₀ were very similar. Compare to an azadirachtin-based commercial product Boursier et al. (2011) attributed the similar performance of the traditional seed-based water extraction to the presence of other components among which terpenoids. Such easy to made botanical extract with a good insecticidal performance can be prepared throughout the year by the producers themselves in developing countries, where synthetic insecticides are not affordable to growers.

Further works are needed to evaluate storage capacity as plant extracts may lose their activity when having a too long contact with water (Schmahl et al., 2010) and chemical degradation of azadirachtin and derivative limonoids increases with temperature (Barrek et al., 2004) and sunlight exposure (Caboni et al., 2009). However, this work underlines that a well-known botanical insecticide that could be part of integrated pest management strategies in developing countries needs that researchers refocus their attention on its development and application.

Conclusion

Neem leaves extracts tested, reduced survival and reproductive potential of the green peach aphid *M. persicae*. There induced mortality of nymphs throughout ingestion. These extracts showed interesting aphicide properties to *M. Persicae* with dose - response relationships well correlated which were observed. Dual-choice assays showed aphids rejection of neem extracts at some concentrations. EPG monitoring showed delayed phloem phase (salivation and ingestion phases), and reduced salivation and ingestion phases indicating repellent properties perceived within the plant tissues and antifeedant properties perceived in the phloem sap. This work reports that neem leaves-based preparations showed aphicidal (antibiosis) and antifeedant behavior

(antixenosis) properties and was efficient to protect vegetable against Sternorrhynca pest *M. persicae*.

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Conflict of Interests

The author(s) have not declared any conflict of interests.

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