Artigo original

DOI: 105902/2236117014888
Revista do Centro de Ciências Naturais e Exatas – UFSM Santa Maria
Revista Eletrônica em Gestão, Educação e Tecnologia Ambiental
e-ISSN 2236 1170 - V. 19, n. 1, jan.- abr. 2015, p. 202-211

REGET/UFSM
Revista Eletrônica em Gestão, Educação e Tecnologia Ambiental

The neem oil in passion fruit management

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Abstract

Brazil is the largest producer of yellow passion fruit; however declined in production is due to diseases caused by Passion fruit Woodiness Virus (PWV) and Cowpea Aphid-Borne Mosaic Virus (CABMV). Another problems faced by industry are the high water and quality loses and thus, reduce the life of the fruits. The aim of this study was to verify the efficacy of natural neem oil in PWV/CABMV managements, and post-harvest characteristics of fruits and the effect of neem cake on the growth of passion fruits plants. In this study, the yellow passion fruit (Passiflora edulis Sims f. Flavicarpa Deg.) of Afruvec selection, was subjected to fertilization by neem cake and sprayed with neem oil at 0.5%. The vegetative growth, stem diameter, and the incidence of the typical symptoms of PWV/CABMV were evaluated. The effect of neem on the fruits in postharvest was evaluated by water loss, firmness of fruits, and chemical analysis was carried as ºBrix, pH and Total Titratable Acidity. The results show that neem oil has great potential to control the incidence of virus in passion fruit, due to the action of repellence. The best result was in the group with oil application every 15 days, resulting up to 42% of plants with symptoms at 162 days after planting, whereas 55% to 65% of control plants with symptoms. In growth and post-harvest, neem cake and neem oil showed no significant effects.

***Keywords**: Organic agriculture. Yellow passion fruit. Neem oil. Neem cake. Sustainability in agriculture*

Submitted: 18/07/2014 - Accept Submission: 09/03/2015

I INTRODUCTION

Brazil is the largest producer of yellow passion fruit; its production grew between 1990 and 1996 from 317,200 to 409,500 tons, fell to 330,800 tons in 2000. Later in the years 2003 and 2004, there was a stabilization of 485,300 and 491,600 tons respectively (IBGE 2007). The main cause of the variation was due to the endemic Passion fruit Woodiness Virus (PWV).

Viruses of various groups can infect passion fruit: Potyvirus, Cucumovirus, Tymovirus, Rhabdovirus, Nepovirus and Geminivirus, but the PWV, from Potyvirus group, is the dominant and causes serious damage (ROSSI, 2006; YUKI et al., 2006). Besides PWV, studies showed that CABMV (Cowpea Aphid-Borne Mosaic Virus) also causes hardening of fruit (ZERBINI et al., 2006), and perhaps this is more related to the main disease (NASCIMENTO et al., 2006). The aphids of the genera *Aphis* and *Myzus* transmit that viruses (BARBOSA; SANTOS FILHO, 2002; DI PIERO et al., 2006; FISCHER; KIMATI; REZENDE, 2005) consequently, deformation occurs in the leaves and fruit, reducing the quality and vigor of the plants, leading to both productive and economic decline (BARBOSA; SANTOS FILHO, 2002; LIMA, 2002; OLIVEIRA, 2006; ROSSI, 2006). The management adopted is very important, however if had a factor to delay or to eliminate the spread of the virus, it would be very important.

Currently there are no permanent and effective proceedings for this viral disease, but there are several recommendations to increase the life of the orchard and to reduce the damage. Such as the use of healthy seedlings, elimination of old and abandoned orchards before the start of production, care on pruning operations to prevent mechanical transmission of the virus, and eradication of plants with symptoms of the virus in seven months after seedlings transplanting in the field (REZENDE, 2006).

However, alternative economically viable and environmentally correct to prevent the spread of the virus is needed (TANG; WEATHERSBEE III; MAYER, 2002; VIEGAS JUNIOR, 2003). The neem, *Azadirachta indica* (A. Juss), is a Meliaceae originated from India with various types of limonoids as secondary metabolites, and azadirachtin is the main (MARTINEZ, 2002; MORDUE; NISBET, 2000; VIEGAS JUNIOR, 2003). It has effect on insect pests, changing their feeding, reproduction and growth, and may have the effect of repellence (HUNTER; ULLMAN, 1992; MARTINEZ, 2002; MORDUE; NISBET, 2000).

Weathersbee III and McKenzie (2005) observed a small but significant effect of repellence against psyllids adults (*Diaphorina citri*) in plants treated with neem, but no preference between treated and non-treated plants for oviposition was identified. The same authors also consider that the application of azadirachtin can be low in concentration to have a management of this pest, and recommend the inclusion of neem in pest management for citrus cultivation.

Myzus persicae nymphs fed with neem extracts and azadirachtin had their endosymbiotic bacteria populations changed, as they have relation to the transmission of Potato Leafroll Virus, consequently its transmission was inhibited (VAN DER HEUVEL et al., 1998). Another vector of viruses, the aphid *Aphis gossypii*, exposed to aqueous extract of neem seed, had increasing mortality, reducing survival time and reducing fertility of their nymphs (SANTOS et al., 2004).

Azadirachtin also has low toxicity to natural predators and pollinators, and it degrades rapidly in the environment (LOWERY; ISMAN, 1995; MARTINEZ, 2002; TANG; WEATHERSBEE III; MAYER, 2002; WEATHERSBEE III and MCKENZIE, 2005). It has antifeedant action, turning food of insect deterrent (MORDUE; NISBET, 2000). It can block Prothoracicotropic hormone, which stimulates the production of ecdysone, thus causing interference in insect molting and therefore its death. Besides affecting the synthesis and release of ecdysteroids, other classes of hormones may also be affected (MORDUE; NISBET, 2000).

Works with various concentrations of neem extract caused deaths in the larval stage of the tomato leaf miner (TRINDADE et al., 2000). At concentration of 4%, the Dalneem® caused adult mortality significantly higher than the control in *O. poecilus* (PINHEIRO; QUINTELA, 2010). Azevedo et al. (2005) noted that treatments with neem oil and pyroligneous acid were the most effective natural insecticides against *Bemisia tabaci* in greenhouse; while neem oil was the best in the field.

In addition to production, another important factor is the preservation of the fruits of passion fruit, because it is a climacteric respiratory pattern with high ethylene released during ripening (DURIGAN; DURIGAN, 2002). Therefore, after harvest, fruit began to loss water and weight,

becoming shrivel and wrinkled, thus it becomes unacceptable during sensory evaluation for consumers (SHIOMI et al., 1996). Therefore, Salomão (2002) recommends the fruits go to market quickly. Studies with fruits treated with wax-based emulsions and emulsion anionic carnauba showed little sign of wilting after 21 days at 25ºC. Other studies recommend the use of polyethylene plastic film attached to the refrigerated environments (SIGRIST, 2002). The use of neem oil in lemon showed that this could inhibit respiration resulting in a lower mass loss (VERMA; DASHORA, 2000).

The passion fruits are targets of fungal, bacterial and viral diseases. The most important are anthracnose, septoria-leaf-spot, and scab (FISCHER; KIMATI; REZENDE, 2005; SIGRIST, 2002). The neem has a great potential to controlling these diseases, Amadioha and Uchendu (2003) recommend the use of neem extract for controlling *Fusarium solani* in storage tomato fruit. In vitro tests the ethanol extract of neem shows ability to inhibit growth and development of mango anthracnose, *Colletotrichum gloeosporioides* (GREENOUGH; TUDELA, 1999), the mycelium of *Phytophthota* ssp. (RAMOS et al., 2007), turning azadirachtin a potential antifungal (GOMES et al., 2007).

For plant development, neem cake seed is also an excellent fertilizer and releases 56.6% nitrogen at 60 days (KOUL; ISMAN; KETKAR, 1990). It has also been reported as a protective effect against nematodes, due to toxic substances released through volatilization, exudation, leaching and decomposition (AKHTAR, 2000), then reducing nematodes population (KHAN; ALAM; AHMAD, 1974). In tobacco, which was incorporated neem cake, there was an increase in the production, even on infected soil with *M. incognita* (DESAI et al., 1979).

Various concentrations of neem over the development of earthworm, which is essential for soil fertility (MAYILSWAMI; REID, 2010), cause no negative effect at high concentration and a slight increase in the development of the worms. Although neem in commercially recommended concentration of extract, do no harm earthworms (*Pheretima peguana*), but extremely high concentrations of neem becomes cytotoxic to them (MUANGPHRA; GOONERATNE, 2011).

The objective of this study was to verify the efficacy of neem oil in the PWV/CABMV management and in the characteristics of post-harvest, besides the effect of neem cake on the growth of passion fruit.

2 MATERIALS AND METHODS

2.1 THE EXPERIMENT

The fieldwork was conducted at a commercial orchard with drip irrigation of yellow passion fruit (*Passiflora edulis* Sims f. flavicarpa Deg.). The conduction system was simple trellis with a smooth wire at 1.9 m high. The spacing used was 2.5 m between plants. The soil where we conducted experiments was red yellow latosol (Oxisol) and we applied good practices listed by Rezende (2006) on field. We used Napier grass as a windbreak for orchards.

We used a randomized block design with three replications in a factorial 3 x 3 in split plot; it was three doses of neem cake and two frequencies of neem oil application and a control (no application). Each plot consisted of four plants amounting 12 plants per treatment.

Seedlings of *Passiflora edulis* Sims f. flavicarpa Deg. were planted in holes on 24/08/2006 with 0, 50 and 100g of neem cake and after 15 days began the spraying of commercial neem oil "Organic Neem" at 0.5% over one third of the each neem cake treatment. In the other third, the spraying occurred every 30 days, and the others were not sprayed. The completion of spraying was the beginning of the harvest - January / February 2007. We sprayed with manual spraying.

We conducted the analysis of post-harvest at the Laboratory of Plant Physiology and Horticulture with selected fruit of passion fruit, donated by an enterprise from Bauru (not from previous preharvest treatments). The fruits were on Stage 2 of maturity, this mean the color of bark surface was yellow-green (50% green and 50% yellow).

For the design, we used four fruits per polystyrene tray and four replications, amounting 16 fruits per treatment, totalizing 80 fruits. The fruits trade classification 1A/2A (with diameter ranging from 65mm up to 85mm) were treated with 0, 1, 2, 3 and 4% of commercial neem oil solution "Organic Neem". Therefore, 5 mL of the solution was applied on each fruit and then

placed in a polystyrene tray. These were stored in a place with an average temperature of 27 º C and relative humidity of 55%.

2.2 PARAMETERS EVALUATED

Vegetative growth: we evaluated the development of shoots by tape measure, measuring from base to apex of the stalk. Stem diameter was evaluated at 30 cm of soil with digital caliper Mitutoyo CD-20.

Plants infected by the vectors: regarding the incidence of viruses, we evaluated through visual symptoms on plants in early harvest on 16/01 and 02/02/2007.

The effect on Neem oil in Postharvest: at the beginning of treatment, we evaluated:

1) TSS (Total Soluble Solids) with digital refractometer in ºBrix;
2) pH with a digital pH meter;
3) TTA (Total Titratable Acidity) according to the methodology of the Institute Adolfo Lutz (INSTITUTO ADOLFO LUTZ, 2005); and
4) Turgidity of fruits, with flattening technique that measures the firmness dependent of cell turgor pressure (CALBO; NERY, 1995).

Each tray was weighed in commercial electronic balance at every 2 morning days until the end of treatment (8 days after application). Every 2 days were also given visual score according to Mota (1999). At the end, we assessed weight, TSS, pH and TTA.

3 RESULTS AND DISCUSSION

3.1 PLANT GROWTH

The heights of the plants treated with neem cake showed no significant difference with the control at 55 days after planting (Table 1). Regarding the diameter of the stem there was no significant difference between the treatments with neem cake and control at 55, 81 and 112 days after planting. However, other studies showed that neem can improve the growth of plants. The application of neem cake in soil increased the concentration of nitrogen in leaves of peach (*Prunus persica* L.), and increased plant biomass compared to control trees (TOSELLI et al., 2010). In chickpea the neem cake reduced nematodes and improved plant growth, increasing water absorption and root nodulation (ANVER; ALAM, 2000). However, the filter cake along with neem oil applied to soil in the cultivation of cane sugar was not effective against the population of nematodes (*Pratylenchus brachyurus*) and sometimes had larger populations than the control (OLIVEIRA et al., 2005).

3.2 INCIDENCE OF FRUIT WOODINESS VIRUS

In Figure 1 we can notice a large increase in the number of contaminated plants at 145 to 162 days after planting, showing the high capacity of virus spread by aphids. The best result was in the group with application every 15 days, with up to 42% of plants infected at 162 d.a.p.; probably due to the short time in which the plants were unprotected, because the azadirachtin has a rapid degradation (TANG; WEATHERSBEE III; MAYER, 2002; WEATHERSBEE III; MCKENZIE, 2005).

Plants that received application every 30 days, also had a good protection against aphids, this mean, up to 50% of contamination at 162 d.a.p., showing some delay of virus spread comparing to control plants, which had 55 % to 65% contaminated plants by the virus at 162 d.a.p.. Although there was no significant difference between treatments, the results showed a trend of protection over neem treated plants from virus dissemination.

The lower viruses' incidence in the treated plant may be due to repellence (WEATHERSBEE III; MCKENZIE, 2005) or the effectiveness of the product containing azadirachtin against aphids (COSTA et al., 2010). For future studies, it could interact neem with other allelochemicals to enhance the effect of repellence or aphids' death (FAROOQ et al., 2011).

Table 1 - Average height (m) at 55 days after planting (d.a.p.) and average diameter (mm) of the stem at 55, 81 and 112 d.a.p. of passion fruit plants submitted to different treatments.

Treatments	Height (m)	Diameter (mm)		
	55 d.a.p.	55 d.a.p.	81 d.a.p.	112 d.a.p.
1. Control	1.45 a	6.32 a	8.29 a	12.29 a
2. Neem oil at 0,5% each 15 days	1.44 a	6.08 a	8.16 a	12.05 a
3. Neem oil at 0,5% each 30 days	1.73 a	6.26 a	8.70 a	13.11 a
4. 50 g of neem cake	1.42 a	5.94 a	8.14 a	12.30 a
5. 50 g of neem cake + neem oil at 0,5% each 15 days	1.56 a	6.47 a	8.91 a	13.26 a
6. 50 g of neem cake + neem oil at 0,5% each 30 days	1.57 a	6.29 a	8.81 a	13.08 a
7. 100 g of neem cake	1.65 a	6.14 a	8.53 a	12.98 a
8. 100 g of neem cake + neem oil at 0,5% each 15 days	1.50 a	6.63 a	8.88 a	12.94 a
9. 100 g of neem cake + neem oil at 0,5% each 30 days	1.54 a	6.14 a	8.26 a	12.60 a

Means followed by the same letter do not differ significantly by Tukey test ($p<0.05$).

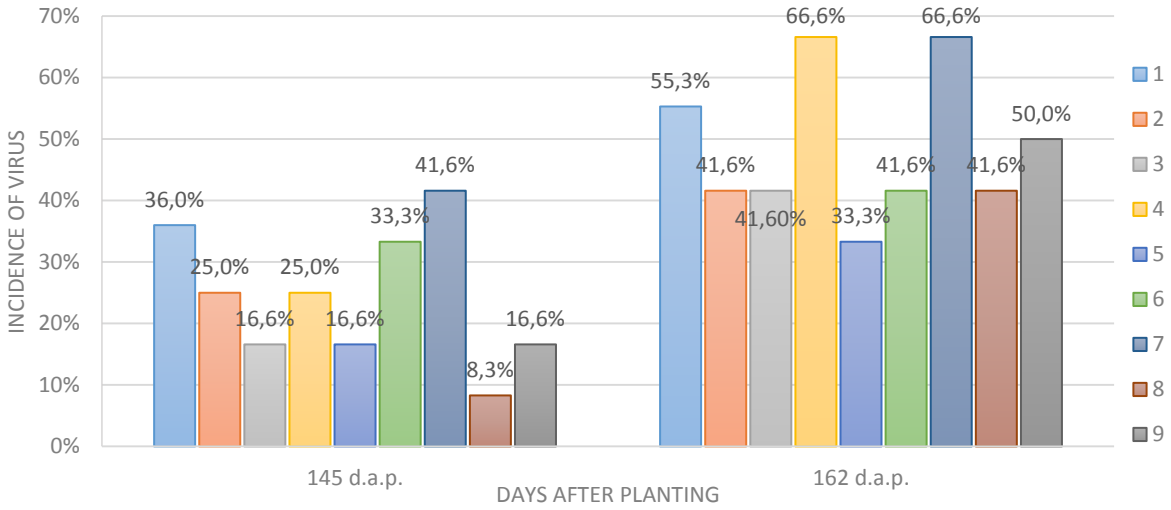

Figure 1 - Incidence in percent (%) of virus in the treatments at 145 and 162 days after planting (d.a.p.).
Labels: 1: control; 2: 0 g of neem cake and leaf application of neem oil at 0.5% every 15 days; 3: 0 g of neem cake and leaf application of neem oil at 0.5% every 30 days, 4: 50 g of neem cake and no application of neem on leaf, 5: 50 g of neem cake and leaf application of neem oil at 0.5% every 15 days, 6: 50 g of neem cake and leaf application of neem oil at 0.5% every 30 days, 7: 100 g of neem cake and no application of neem on leaf, 8: 100 g of neem cake and leaf application of neem oil at 0.5% every 15 days, 9: 100 g of neem cake and leaf application of neem oil at 0.5% every 30 days. No-significant differences between treatments were observed.

3.3 POSTHARVEST

The passion fruit is a climacteric fruit so has high level of water loss (SHIOMI et al., 1996), as can be seen in the averages of water loss (Table 2), but there was no difference between neem treatments and control. However, Verma and Dashora (2000) showed that application of neem (4%) and diphenyl (500 mg/kg per fruit) in lemon inhibits the physiological weight loss. Therefore, it is necessary further studies on the protection of passion fruit against high level of water loss.

Table 2 - Average water loss (%) of passion fruit, treated with different concentrations of neem oil on day 0 (control) and 2, 4, 6 and 8 days after application (d.a.a.) of the oil.

Treatments	day 0	2 d.a.a.	4 d.a.a.	6 d.a.a.	8 d.a.a.
Control	0 a	3.75 a	5.06 a	7.31 a	7.88 a
1% of neem	0 a	2.81 a	4.44 a	6.75 a	7.62 a
2% of neem	0 a	2.62 a	3.75 a	6.00 a	7.31 a
3% of neem	0 a	1.50 a	3.56 a	6.00 a	6.38 a
4% of neem	0 a	2.62 a	4.31 a	6.75 a	7.69 a

Means in the same column followed by the same letter do not differ significantly by Tukey test ($p<0.05$).

As for fruit firmness (Table 3) there was no significant difference between treatments.

Regarding chemical evaluations, it is observed that the passion fruit is, in most treatments, slightly more acidic over the following days, but the neem oil does not interfere on pH (Table 4). Total Soluble Solids (TSS) was not significantly different between treatments and control (Table 4). Verma and Dashora (2000) found lower values of TSS in lemons treated with neem (4%) on evaluated days. As for Total Titratable Acidity (TTA) of treatments 1, 2 and 3% neem oil on the day 8 after administration were significantly lower compared to day 0. However, there was no significant difference between treatment and control on day 8 (Table 4). The ratio between TSS and TTA had no significant difference between treatments, but the treatments 1 and 2% had a significantly higher ratio when comparing day 0 and day 8 after treatment application (Table 4). However, Wijewardane and Guleria (2013) found different results in apple fruit submitted to 1.5 and 2% of neem oil with pre-cooling; these treatments showed that fruits retained the best physical and chemical characteristics, and they reduced significantly the incidence of diseases.

Table 3 - Average firmness (kg/cm2) for passion fruit, treated with different concentrations of neem oil on day 0 (control) and 8 days after application (d.a.a.) of the oil.

Treatments	day 0	8 d.a.a.
Control	8.80 Aa	2.65 Ba
1% of neem	8.80 Aa	2.29 Ba
2% of neem	8.80 Aa	2.83 Ba
3% of neem	8.80 Aa	2.43 Ba
4% of neem	8.80 Aa	2.79 Ba

Means in the same row followed by the same capital letter do not differ significantly by Tukey test ($p<0.05$). Means in the same column followed by the same small letter do not differ significantly by Tukey test ($p<0.05$).

Table 4 - Average pH, Total Soluble Solids (TSS in °Brix), Total Titratable Acidity (TTA) and TSS/TTA ratio for passion fruit, treated with different concentrations of neem oil on day 0 (control) and 8 days after applying (d.a.a.) the oil.

Treatments	Ph		TSS		TTA		TSS/TTA	
	day 0	8 d.a.a	day 0	8 d.a.a	day 0	8 d.a.a	day 0	8 d.a.a
Control	3.12 Aa	3.06 Aa	15.70 Aa	14.6 Aa	0.39 Aa	0.30 Aa	39.50 Aa	49.10 Aa

1% of neem	3.12 Aa	3.07 Aa	15.70 Aa	14.1 Aa	0.39 Aa	0.27 Ba	39.50 Aa	56.02 Ba
2% of neem	3.12 Aa	3.14 Aa	15.71 Aa	14.5 Aa	0.39 Aa	0.24 Ba	39.51 Aa	60.21 Ba
3% of neem	3.12 Aa	3.05 Aa	15.71 Aa	13.7 Aa	0.37 Aa	0.28 Ba	39.50 Aa	48.91 Aa
4% of neem	3.12 Aa	3.04 Aa	15.70 Aa	14.4 Aa	0.39 Aa	0.32 Aa	39.51 Aa	46.60 Aa

Means in the same row followed by the same capital letter have no significant differences by Tukey test ($p < 0.05$). Means in the same column followed by the same small letter have no significant differences by Tukey test ($p < 0.05$).

4 CONCLUSIONS

From the results obtained, we can conclude that:

1. The neem oil has great potential to control the incidence of virus in passion fruit, due to its repellence action on the vector insect, demonstrating a lower incidence of symptoms of the virus in treated plants. Future research can integrate the neem with other allelochemicals and verify the enhancement of its effect.

2. Concerning to post-harvest, neem oil had no significant effect on the life of the fruit to market in natura, the same we can say about the effect of neem cake on the vegetative growth, thus more studies are necessary in these subjects both in laboratory and field. It is interesting for future studies to evaluate fruit characteristics using fruit from treated plants with neem and disease incidence.

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