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Mortality and adult deformation caused by vegetable oils sprayed on *Dione juno juno* (Cramer, 1779) larvae

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ABSTRACT

The management of *Dione juno juno* (Cramer, 1779) (Lepidoptera: Nymphalidae) larvae in passion fruit has been done almost exclusively with synthetic insecticides. The development of alternative methods is desirable to reduce problems caused by the exclusive use of chemical control. Vegetable oil from cotton seeds (*Gossypium hirsutum* L.), castor bean (*Ricinus communis* L) oil, and neem (*Azaditachta indica* A. Juss) were tested against larvae of *D. juno juno*, to evaluate mortality of larvae and deformities in adult insects. Treatments included spray solutions with 2.0% of oils (emulsified with detergent). Detergent (1.0%) and distilled water were used as controls. Experiments were conducted for second, third and fifth instar larvae. Evaluations were performed one and five days after treatment. In the three instars tested, neem oil showed higher percentage of mortality (83. 73 and 64% respectively) than other treatments. Castor bean and cotton oils are more efficient on second and third larval instar. Spraying of either oil determined deformation in adults. These deformations were more drastic in the insects which larvae were treated with cotton seed oil than neem and castor bean oil.

Key words: alternative control, passionflower, ricinoleic acid, gossypol, azadirachtin.

INTRODUCTION

Passion fruit (*Passiflora edulis* Sims) is a widespread tropical plant largely consumed *in natura* or as juice, being also used by pharmaceutical and cosmetic industries. Phytosanitary problems, including pests and diseases, are important aspects of crop production. Among the arthropods, the leaf-chewing caterpillar *Dione juno juno* (Cramer 1779) (Lepidoptera: Nymphalidae) is considered a key pest due to the damages it causes and the frequency it occurs (Lara et al., 1999; Angelini and Boiça Júnior 2007).

Larvae of *D. juno juno* measures 1.5 mm length (yellowish) when newly-hatched and 20-35 mm (dark brown color) at the final of the larval period and presents dark head and spines on the body (Gallo et al., 2002). Larvae have gregarious behavior up to the 5th instar and close to the pupal period, searching for places to fasten (Boaretto et al., 1994). Initial damage is insignificant due to low the leaf consumption by the small size insects. However, as the insect grows, leaf consumption increases. Considerable reduction of foliar area is observed and total yield losses may occur (Boiça Júnior et al., 2008). Chemical control is the standard strategy used to manage this pest in the field. An imediate side effect caused by insecticides is the elimination of the pollinators, mostly bumblebees, which reduces yields (Boiça Júnior et al., 1999). Side effects for pollinators are particularly severe by neonicotinoids insecticides, including severe reduction of bumblebees colony growth and queen reproduction (Whitehorn et al., 2012). Furthermore, reduction of natural enemies, environmental contamination and workers' toxicological hazards associated with the utilization of synthetic chemical insecticides. Passion fruit is cultivated mostly by small farmers, and, in general, is done under limited conditions to deal with pesticides (personal protective equipments, tractors, sprayers etc).

In addition, production cost reduction is of concern and justifies the search for less aggressive and cheaper methods of manage pests. Neem (*Azadirachta indica* A. Juss) oils have been used worldwide to manage several plant arthropod pests and diseases (Schmutterer 1990). Besides the neem oils, the castor bean (*Ricinus communis* L.) oil, which is rich in ricinoleid acid, has been tested for pest control in some crops (Lins Jr et al., 2007; Pacheco et al., 1995). Similarly, cotton (*Gossypium hirsutum* L.) oils, which are a mix of triacylglycerols (Salgado et al., 2003), contain gossypol, an anti-nutritional phenolic that provides plant resitance against pathogens and arthropod pests (Macedo et al., 2007). Thereby, vegetable oils obtained from pressing cotton and castor been seeds were also tested against first, second and fifth larvae of D. *juno juno*, to assess larvae mortality, viability and deformation in adults of larvae previously treated with oils.

MATERIAL AND METHODS

Insects were collected in a field cultivated under the organic farming system, in Ortigueira, PR (24°12'18"S e 50°56'56"O). Leaves containing larvae were brought to the laboratory, grouped by instar (2nd, 3rd and 5th) and placed in acrylic boxes containing passion fruit leaves as food.

Treatments included cotton, castor bean and neem oils (2%). Cotton and castor bean seeds oils were emulsified using neutral detergent (Alpes®) (1%). Neutral detergent solution (1%) (blank) and distilled water (control) were also used. Neem oil was used in an emulsified commercial formulation (Azamax®). Spray solutions were prepared and sprayed on larvae in acrylic boxes (11.0 x 11.0 x 3.5 cm) using an airbrush (Passehe – 147493) coupled to a compressor aspirator (Fanem – Diapump®, Model: 089 – Cal) adjusted to 10 BAR.

Six, eight and six larvae per box were used for the second, third and fifth instar, respectively. 100 μL of solution was applied per pot to the second and third instars and 200 μL to fifth instar. After spraying, pots with treated larvae were maintened in environmental chambers (25 ± 2ºC, 70% ±10% UR) until adult emergence. Mortality was assessed one and five days after spraying (das). Occurrence of deformations was assessed after adult emergence.

A completely randomized design was used with six replicates. Analysis of variance was achieved by Kruskal-Wallis test and means compared by the Student-Newman-Keuls test ($p<0.05$).

RESULTS AND DISCUSSION

In the assay with the first instar larvae, during the first assessment (1 das), higher mortality was observed in treatments with detergent or oils compared to the control (Table 1). In the second assessment (5 das), mortality was also higher for treatments with oils and detergent than the control, except for the castor bean oil treatment (Table 1).

Table 1. Accumulated mortality (% ± SE) of 2nd instar larvae of *Dione juno juno* after spraying neem, cotton and castor bean oils (2%) in laboratory.

Treatments	Number of dead larvae	
	1das	5das
Control (water)	3 (± 0,40) b[1]	11 (± 0,81) c
Water + detergent (blank)	42 (± 2,16) a	55 (± 1,75) b
Neem oil	25 (± 0,54) a	83 (± 1,26) ab
Castor bean oil	25 (± 0,54) a	36 (± 1,47) bc
Cotton oil	36 (± 1,16) a	69 (± 1,98) ab

[1]Means followed by different letter in the same collum are different by Student-Newman-Keuls test at 5%.

In the second assay (third instar larvae), during the first assessment (1 das), mortality was similar among treatments (Table 2). In the second evaluation (5 das), insects treated with oils showed higher mortality than the control. Neem, castor bean and cotton oils treatments caused 73, 40 and 44% of mortality, respectively.

Table 2. Accumulated mortality (% ± SE) of 3nd instar larvae of *Dione juno juno* after spraying neem, cotton and castor bean oils (2%) in laboratory.

Treatments	Number of dead larvae	
	1das	5das
Control	0 (± 0,00) a[1]	2 (± 0,40) c
Water + detergent (blank)	4 (± 0,51) a	14 (± 0,75) bc
Neem oil	10 (± 0,98) a	73 (± 1,47) a
Castor bean oil	21 (± 1,63) a	40 (± 1,83) ab
Cotton oil	17 (± 1,03) a	44 (± 0,54) ab

[1]Means followed by different letter in the same collum are different by Student-Newman-Keuls test at 5%.

For the 5th instar larvae, similar mortality among treatments was found for the first assessment (1 das). During the second assessment (5 das), neem oil caused higher mortality than other treatments (Table 3) and similar mortality was found among other treatments.

Pupae, which larvae were previously treated with oils, presented a softened aspect when compared with pupae which larvae were previously treated with distilled water and detergent. Adults from larvae treated with distilled water and detergent presented normal external apperance without deformation and 100% of emergency. In all adults (100%), whose larvae were treated with oils, deformations were observed.

Adults, whose larvae were treated with neem oil, presented wrapped upper wings and lower wings absent or in different number. These emerged butterflies have just one pair of upper wings and either none or just one lower wing (Fig. 1a). Butterflies from larvae treated with castor bean oil treatment presented deformation in the upper wings and absence of lower wings, showing also wrapped or small scale wings (Fig. 1b). Insects whose larvae were treated with cotton oil, the effects on pupae and adults were visually even more severe. Adults had a lot of difficulty to release themselves from the pupal enclosure. When they succeed in emerging, wings were softened and deformed (Figure 1c).

Table 3. Accumulated mortality (% ± SE) of 5nd instar larvae of *Dione juno juno* after spraying neem, cotton and castor bean oils (2%) in laboratory.

Treatments	Number of dead larvae			
	1DAA		5DAA	
Control	11 (± 0,52)	a[1]	17 (± 0,63)	b
Water + detergent	13 (± 0,75)	a	17 (± 0,63)	b
Neem oil	16 (± 0,89)	a	64 (± 1,17)	a
Castor bean oil	19 (± 0,75)	a	19 (± 0,75)	b
Cotton oil	22 (± 1,03)	a	25 (± 1,22)	b

[1]Means followed by different letter in the same collum are different by Student-Newman-Keuls test at 5%.


(a)


(b)


(c)

Figure 1. Newly hatched *Dione juno juno* butterfly which larvae were previously treated with neem oil (a), castor beans oil (b), and cotton oil (c).

Mortality caused by neem oil is probably due to the toxic effect of azadirachtin, an oxygenated terpene considered toxic to a wide range of arthropod pest (Schmutterer 1990). Azadirachtin has been reported as having strong activity on chewing insects, without causing immediate death of the insect after ingestion (Martinez 2002). The results reported here, with significant mortality in the second assessment, may be associated with the growth regulator effect. This effect was also characterized for *Ephestia kuehniella* Zell (Lepidoptera: Pyralidae) moth, ladybird *Epilachna varivestis* Muls. (Coleptera: Coccinelidae) and honeybee *Apis mellifera (Himenoptera: Apidae) (Rembold et al., 2009).* Similar results were also obtained for the last instar of *S. exigua* (Viñuela et al., 2000) and the third instar of *S.litura* (Johnson et al., 2003), using commercial neem formulations and seeds extracts, respectively.

Pupae and adult deformations in insects treated with neem oil (Fig. 1) are probably due to ingestion of azadirachtin which causes malfunction of the molting process. Pupae malformation and adult insects with defective wings were also previously reported *(*Schmutterer 1990). The deleterious effects of azadirachtin on the molting pupae-adult, as observed here, have also been reported for *E. kuehniella (Hymenoptera, Apidae) (Rembold et al., 2009).* The azadirachtin mimics the ecdises hormone and disturbs its action, affecting molting and growing (Martinez 2006), as previously found for larvae of Coleoptera, Lepidoptera, Hymenoptera and Diptera (Martinez 2002).

Cotton oil bioactivity in insects, as observed in our study, has been attributed mostly to gossypol, an aldehyde terpene that inhibits proteinase and interferes with the process of protein degradation in the insect midgut, reducing amino acids availability and, consequently, imparing protein synthesis for growing, developing and reproducing (Macedo et al., 2007). These inhibitors may also affect insect development indirectly, via feedback mechanism, which leads to an increased production of digestive proteinases to compensate for the low levels of amino acids available. These would be displaced for proteinases syntheses over other essential proteins (Macedo et al., 2007).

Insecticide effects of castor bean oil observed in this study corroborates with previous reports. Bioactivity against cotton boll weevil (*Anthonomus grandis* Boh) (Coleoptera: Curculionidae), together with neem oil, was observed in the field (Lins Jr et al., 2007). Castor bean oil also protected stored garbanzo beans (*Cicer arietinum* L.) from *Callosobruchus maculatus* (F.) and *C. phaseoli*em (Coleoptera: Bruchidae) beetles. This insecticide activity has been attributed to triglycerides and oleic acids. Some authors have suggested that oil affects the tracheal system, causing difficulties or blocking insect respiration.

Our results suggest the eventual use of castor and mostly cotton oil in *D. juno juno* management in the field. In general, passion fruit is cultivated by small fields. Although oils were used in relatively high concentrations (2%), their low costs compensate for the high amount of oil per area. Application of the oil on early instars of the pest is fundamental since mortality decreases with increasing instars.

Previous studies showed that cotton oil and castor bean oil showed synergism when applied with biopesticides (El-Raffez et al., 2013; Rondelli et al., 2011) or chemical insecticides (Tembo and Murfitt 1995; Wanyika et al., 2009). Enhancement of microbial insecticides by mixing vegetable oils reduces pest control costs and environmental contamination (El-Hafez et al., 2013). According to these authors, when added to the spray soup, oils decrease loss of insecticides by diminishing drift and increasing adhesion, wetting, spreading and depositing on plant leaves, protecting spores from UV radiation oils. Entomophatogenic organisms for *D. juno juno* includes *Bacillus thuringiensis* (Villani et al., 1980) and polyedrosis virus (Andrade and Habid 1984). Further investigations could be done to evaluate the effect of mixing cotton and castor bean oils to these biopesticides to evaluate an eventual efficiency improvement.

CONCLUSIONS

Neem oil caused mortality when applied on second, third and fifth instars of *D. juno juno.* Cotton oil caused an intermediate level of mortality, when applied on second and third instars. Castor bean oil also had effects on second and third instars. Adult malformation and consequent inviability were found for the three oils; however, they were more drastic when larvae were treated with cotton oil.

REFERENCES

Andrade CFS and Habid MEM (1984) Natural occurrence of baculoviruses in populations of *Heliconini* (Lepidoptera: Nymphalidae) with symptomatological notes. Revista Brasileira de Zoologia 2: 55-62.

Angelini MR and Boiça Júnior AL (2007) Preferência Alimentar de *Dione Juno Juno* (Cramer, 1779) (Lepidoptera: Nymphalidae) por genótipos de maracujazeiro. Revista Brasileira de Fruticultura, 29: 276-281.

Boaretto MAC, Brandão ALS and São José AR (1994) Pragas do maracujazeiro. In: São José AR (ed.) Maracujá: produção e mercado. DFZ-UESB, Vitória da Conquista, p. 99-107.

Boiça Júnior AL, Lara FM and Oliveira, JC (1999) Flutuação populacional de *Dione juno juno* (Cramer, 1779) (Lepidoptera: Nymphalidae) em maracujazeiros (*Passiflora* spp.), métodos de amostragem e resistência de genótipos. Scientia Agricola 56: 437-441.

Boiça Júnior AL, Angelini MR and Oliveira JC (2008) Aspectos biológicos de *Dione juno Juno* (Cramer) (Lepidoptera: Nymphalidae) em genótipos de maracujazeiro. Revista Brasileira de Fruticultura 30: 101-105.

El-Hafez ABD, Hanan EF, El- Rahim ABD and Mohamed EM (2013) Effect of some vegetable oils in enhancing the potency of bioinsecticides against the cotton leafworm. Journal of Agricultural Research, 91: 1361-1383.

Gallo D, Nakano O, Silveira Neto S, Carvalho RPL, Batista GC, Berti Filho E, Parra JRP, Zucchi, RA, Alves SB, Vendramim JD, Marchini LC, Lopes JR and Omoto C (2002) Entomologia Agrícola FEALQ, Piracicaba, 682p.

Johnson S, Dureja P and Dhingra S (2003). Photostabilizers for azadirachtin-A (a nimbased pesticide). Journal of Environmental Science and Health 4: 451-462.

Lara FM, Boiça JR AL and Barbosa JC (1999) Preferência alimentar de *Dione juno juno* (Cramer) por genótipos de maracujazeiro e avaliação do uso de extratos aquosos. Scientia Agricola 56: 665-671.

Lins JR JC, Nascimento ML, Menezes MAS, Rodrigues IJS, Lima ESA, Dias TKR, Dias PC, Cardoso UP and São José AR (2007). Controle alternativo do bicudo-do-algodoeiro, *Anthonomus grandis* (Coleoptera: Curculionidae). Revista Brasileira de Agroecologia 2: 987-990.

Macedo LPM, Cunha, US and Vendramim, JD (2007) Gossipol: fator de resistência a insetos-praga. Campo Digital 2: 34-42.

Martinez SS (2002) O Nim - *Azadirachta indica*: natureza, usos múltiplos, produção. IAPAR, Londrina, 142 p.

Martinez S.S. (2006) O Nim - Azadirachta indica - um Inseticida Natural. IAPAR, Londrina, 5p.

Pacheco IA, Castro MFPPM, Paula DC, Lourenção, AL, Bolonhezi S and Barbieri MK (1995) Efficacy of soybean and castor oils in the control of *Callosobruchus maculatus* (F.) and *Callosobruchus phaseoli* (Gyllenhal) in stored chick-peas (*Cicerarientinum*L.). Journal of Stored Products Research 31: 221-228.

Rembold H, Sharma, GK and Czoppelt C. (2009) Azadirachtin: a potent insect growth regulator of plant origin. Journal of Applied Entomology 93: 12-19.

Rondelli VM, Pratissoli D, Polanczyk RA, Marques EJ, Sturm GM and Tiburcio MO (2011) Associação do óleo de mamona com *Beauveria bassiana* no controle da traca-das-cruciferas. Pesquisa Agropecuária Brasileira 46: 212-214.

Salgado APSP, Cardoso MG, Souza PE, Souza JA, Abreu CMP and Pinto JEBP (2003) Avaliação da atividade fungitóxica de óleos essenciais de folhas de Eucalyptus sobre *Fusarium oxysporum, Botrytis cinerea e Bipolares sorokiniana*. Ciência e Agrotecnologia 27: 249-254.

Schmutterer H. (1990) Properties and potential of natural pesticides fromthe nim tree, *Azadiracta indica* Annual Review Entomology 35: 271-297.

Tembo E and Murfitt RFA (1995) Effect combining vegetable oil with pirimiphos-methyl for protection of stored wheat against *Sitophilus granarius* (L.). Journal Stored Products Research 31: 77-81.

Villani HC, Campos AR and Gravena S. (1980) Effectiveness of *Bacillus thuringiensis* Berliner and fenitrothion + fenvalerate for the control of the passion-fruit larva *Dione juno juno* (Cramer, 1779) (Lepidoptera, Heliconidae). Anais da Sociedade Entomologica do Brasil 9: 255-260.

Viñuela E, Adán A, Smagghe, G, González M, Medina MP, Budia F, Vogt H and Estal P (2000) Laboratory effects of ingestion of azadirachtin by two pests (*Ceratitis capitata* and *Spodoptera exigua*) and three natural enemies (*Chrysoperla carnea, Opius concolor* and *Podisus maculiventris*). Biocontrol Science and Techonology 10: 65-177.

Wanyika HN, Kareru PG, Keriko JM, Gachanja AN, Kenji GM and Mukiira NJ (2009) Contact toxicity of some fixed plant oils and stabilized natural pyrethrum ex- tracts against adult maize weevils (Sitophilus zeamais Motschulsky). African Journal of Pharmacy and Pharmacology 3: 66-69.

Whitehorn P, O'Connor S, Wackers FL and Goulson D (2012) Neocotinoid pesticide reduces bumble bee colony growth and queen production. Science 20: 351-352.

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