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Association of peanut cultivars and aqueous neem extract in the feeding and development of *Spodoptera frugiperda* (J. E. Smith)

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Abstract. The current study aimed to test how aqueous neem *Azadirachta indica* A. Juss. extract affects the attractiveness, feeding and development of *Spodoptera frugiperda* (J. E. Smith) on different: peanut cultivars. Tests were carried out under laboratory conditions with the cultivars IAC 503 and IAC 147 and aqueous neem extract in concentrations (mass/volume) of 5 and 10%, obtained from seeds and dried in an oven at a temperature of 35 to 38 °C for 15 days, with caterpillars offered a food choice preference test. For this, leaf discs from each treatment were placed in Petri dishes into which three first instar caterpillars were released. To assess resistance biology, single newly-hatched caterpillars were transferred to individual Petri dishes, where the following biological parameters were measured: larval mortality after 1, 3, 5, 7 and 10 days; pupal mortality; total mortality; weight of 10-day-old caterpillars; pupal weight at 24 hours; and adult longevity. The main conclusions of the work, include: cultivar IAC 147 was less attractive and consumed less; cultivar IAC 503 plus 10% neem extract reduced leaf consumption; cultivar type did not affect caterpillar biological development, and 10% neem extract caused 100% mortality in *S. frugiperda*.

Keywords: *Arachis hypogaea; Azadirachta indica;* limonoids; insecticidal plant; resistance.

The peanut (*Arachis hypogaea* L.) is the world´s fourth-ranking grown oil crop, with products being used in human food, mainly for the production of edible oil, confectionery, sweets, pastes, biofuels and fresh *in natura* (Rodrigues *et al.* 2016). The State of São Paulo is responsible for most Brazilian peanut production, with approximately 90% of the national crop (CONAB 2020), in the sugarcane-growing areas of the state, this crop has marked economic importance, as it is used in crop rotation with sugar cane during the off-season, so providing an alternative income during this period.

Fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is a frequent pest of peanuts (Tasso Júnior *et al.* 2004; Gabriel 2016), feeding on leaves and tender stems of plants, with the capacity to cause serious damage if high population densities are reached (Almeida 2013).

Synthetic insecticides are still the method most used for *S. frugiperda* control. However, continuous and indiscriminate insecticide use can cause imbalances in an agro-ecosystem, leaving residues in food, and selecting for resistant populations of insect pests, as well as increasing production costs (Rodrígues-Hernández & Vendramim 1996; Sparks & Lorsbach 2017; Nauen *et al.* 2019). Only five products are registered by the Brazilian "Ministério da Agricultura, Pecuária e Abastecimento" (MAPA) for agricultural management of this crop, these belonging to the chemical groups: benzoylureas, unsaturated acetate and oxime methylcarbamate, as well as the biological control agent *Bacillus thuringiensis* (AGROFIT 2020), thus complicating resistance management for this pest.

The use of resistant cultivars can be considered a viable alternative method for pest control, since it does not cause adverse effects to the agro-ecosystem, reduces the population of the pest below the level of economic damage and is compatible, in general, with other control biological and chemical tactics (Lara 1991). For peanuts, several lines of research have shown promising results; Boiça Junior *et al.* (2013) found that the IAC Caiapó cultivar showed résistance to attack by *Enneothrips flavens* Moulton (Thysanoptera: Thripidae), while Di Bello *et al.* (2015) concluded that IAC 147, IAC Runner 886, IAC 22 and IAC 8112, showed antibiose resistance to *Stegasta bosquella* (Chambers) (Lepidoptera: Gelechiidae). Despite this, studies of resistance of peanut cultivars and their interaction with other control methods are still scarce.

Another promising method in integrated pest management is the use of plants with natural insecticidal properties, the use of which was already common in tropical countries before the development of synthetic insecticides (Ruberto *et al.* 2002; Gahukar 2012), an approach that has given promising results for controlling *S. frugiperda* and other insect pests (Koul *et al.* 2004; Campos & Boiça Junior 2012; Andrade *et al.* 2013) indicating the bioeffectiveness and mode of action of some salanin group limonoids from *Azadirachta indica* A. Juss.,

and their central role in a multicomponent system against lepidopteran larvae.

Among the insecticidal plants, those belonging to the family Meliaceae are prominent, with *A. indica* (popularly known as "neem"), being the most used. This plant has azadiractin (AZ) as the main molecule in the active ingredient. This secondary metabolite is a triterpene limonoid with a strong anti-feedant capacity. In addition, neem contains gedunin from which 6*β*-hydroxygedunin can be derived synthetically; while the limonoids nimbocinol, salannin and azadiradione can also be extracted from *A. indica* (Drijfhout & Morgan 2010; Ayil-Gutiérrez *et al.* 2018).

Neem extracts induce mortality by preventing insects from carrying out ecdysis (Mordue & Nisbet 2000; Góes *et al.* 2003). This occurs via induced mesenteric histolysis (Correia *et al.* 2009; Roel *et al.* 2010), due to the deterrent and/or suppressive factors present in the plant extracts (Carpinella *et al.* 2003; Medeiros & Boiça Junior 2005; Rossetti *et al.* 2008).

Advances in studies involving peanut cultivars and plant extracts are essential to study the possible resistance factors present in such cultivars, to evaluate the insecticidal bioactivity of active compounds present in assayed plant species, and their associated effects, so that they may be used later in an integrated management program against *S. frugiperda* when it attacks cultivated peanuts, as well as other pest species of Lepidoptera.

The current study aimed to evaluate how neem aqueous extract affected the, feeding and development of *S. frugiperda* on different cultivars of peanuts, and whether it altered their attractiveness.

MATERIAL AND METHODS

Experiments were carried out in in the FCAV/UNESP plant resistance laboratory, Jaboticabal-SP, at a temperature of 25 ± 1 ºC, relative humidity of 70 ± 10% under a 14 hours photophase. Ground-creeping peanut seedlings belonging to cultivars IAC 503 and IAC 147, (which are planted commercially in the region), were sown in a greenhouse in 1 L volume plastic bags containing soil, sand and manure in the proportion 2:1:1. To carry out the experiments, a stock of *S. frugiperda* was maintained on an artificial diet following Kasten Junior *et al.* (1978).

To prepare the insecticide extract, neem fruits were collected, seeds were then removed and dried in an oven at 35 to 38 °C for 15 days, until they reached constant weight. They were then ground with a knife mill using a 0.8 mm grade sieve. Suspensions were prepared containing either 5 and 10 g of ground seeds in 100 mL of distilled water, soaking for 12 h. After this, the mixture was filtered through voile tissue, to give extracts in concentrations (mass/volume) of 5 and 10%.

Freshly opened peanut leaflets were collected in a greenhouse from 30-day old plants, and leaf disks of 2 cm in diameter were prepared using a punch. Discs were then immersed in one of the two concentrations for one minute. For the control treatment leaf-discs were immersed in distilled water for one minute. Experiments consisted of six treatments in a 2 x 3 factorial scheme, two cultivars (IAC 503 and IAC 147) and three concentrations (0, 5 and 10%). Leaf discs were placed on paper towels to remove excess moisture, then transferred to Petri dishes containing filter paper moistened with distilled water.

The food choice preference test was conducted in 14 cm diameter Petri dishes containing filter paper moistened with distilled water, with six leaf discs placed equidistantly from each other and close to the edge of the plate, one for each of the treatments. Three first instar *S. frugiperda* caterpillars were released into the center of the Petri dish. Experimental design was a randomized block with 15 repetitions, a repetition being represented by a Petri dish. Attractiveness/repellency of discs to the caterpillars was evaluated across periods of 1, 3, 5, 10, 15, 30, 60, 120, 360, 720 and 1440 minutes after their release.

In addition, consumed mass was recorded. For this, leaf discs representing each treatment were removed in pairs from newly opened and symmetrical leaflets from the peanut plants, then materials were constructed and tests conducted as described above. At test end, leaf discs that had been offered to the caterpillars for the test were placed in an oven at 60 ºC for 24 hours to obtain dry mass.

Studies of larval biology were conducted using newly-hatched stock-bred caterpillars transferred to Petri dishes (one caterpillar per dish). Peanut leaflets treated with aqueous neem extract were offered to the caterpillars first, and when/if they were ingested, only then were control leaflets (those immersed only in distilled water) subsequently presented. This allowed evaluation of product efficiency in a single application. Biological parameters evaluated were: larval mortality after 1, 3, 5, 7 and 10 days; pupal mortality; total mortality (caterpillars + pupae); weight of 10-day-old caterpillars; pupal weight at 24 hours; and adult longevity. For this, a completely randomized design with 30 replications was used.

Results obtained in the tests were submitted to analysis of variance (ANOVA) with an F test, and means compared with a Tukey test, at 5% probability. Data on the number of caterpillars attracted to the various food items at different times and dry mass consumed were transformed using $(x+0.5)^{1/2}$ and the percentage of mortality in arcsine $(x/100)^{1/2}$, following the precepts of normality and homoscedasticity tests.

RESULTS AND DISCUSSION

Based on the results obtained from the food preference test with a chance to choose, there was no significant difference in the attractiveness/repellability of the evaluated cultivars to *S. frugiperda* first instar caterpillars (Table 1). This partly supported the results of Campos et al. (2010), who evaluated the lack of preference by feeding third instar *S. frugiperda* caterpillars for low growth habit peanut cultivars, and found no difference in levels of caterpillar attraction to IAC 503 and IAC 147. Boiça Junior et al. (2013) evaluating *S. cosmioides* caterpillar feeding preferences for different peanut cultivars, including IAC 503 and IAC 147, also reported a lack of significant differences between the cultivars.

There was a significant difference in the capacity of neem extract doses to repel *S. frugiperda* at nearly all times evaluated, the only exception being the evaluation carried out 5 minutes after the release of the caterpillars (Table 1). Dry mass consumed by caterpillars did not differ between evaluated cultivars (Table 1). Campos *et al.* (2010) observed no difference in the leaf area consumed by *S. frugiperda* third instar caterpillars between peanut cultivars in tests both with and without choice.

Dry mass consumed by *S. frugiperda* caterpillars differed from the control (concentration 0) ($P \leq 0.01$) (Table 1), showing control efficiency for both of the two studied concentrations (5 and 10%). In tests with *S. eridani*, Rossetti *et al.* (2008) fed *Lectuca sativa* leaves treated with neem extract at concentrations of 2%, 5% and 10%, and observed a significant reduction in consumption, in tests with and without chance

Table 1. Average number of newly-hatched *Spodoptera frugiperda* caterpillars attracted to peanut leaf discs treated with neem extract for different times and amounts of dry matter consumed, in a chance to choose test under laboratory conditions. Temperature: 25 ± 1 °C; RH: 70 ± 10%; photophase: 14 h.

Cultivars (C)	Minutes[1]											Dry Mass CONSUMED (g)[1]
	1	3	5	10	15	30	60	120	360	720	1440	
IAC 503	0.49 a	0.49 a	0.58 a	0.60 a	0.64 a	0.56 a	0.56 a	0.64 a	0.62 a	0.60 a	0.82 a	0.34 a
IAC 147	0.29 a	0.40 a	0.47 a	0.27 a	0.42 a	0.38 a	0.38 a	0.51 a	0.49 a	0.53 a	0.58 a	0.25 a
F (C)	1.63^{NS}	0.20^{NS}	0.36^{NS}	3.36^{NS}	0.99^{NS}	0.77^{NS}	0.75^{NS}	0.17^{NS}	0.34^{NS}	0.46^{NS}	1.62^{NS}	2.30^{NS}
Neem Extract (N)												
5%	0.37 ab	0.43 ab	0.47 a	0.27 a	0.37 a	0.30 a	0.30 a	0.33 a	0.20 a	0.53 ab	0.67 a	0.24 a
10%	0.17 a	0.17 a	0.37 a	0.13 a	0.27 a	0.13 a	0.13 a	0.20 a	0.27 a	0.37 a	0.20 a	0.21 a
0	0.63 b	0.73 b	0.73 a	0.90 b	0.97 b	0.97 b	0.97 b	1.20 b	1.20 b	0.80 b	1.23 b	0.44 b
F (N)	3.39*	4.89*	2.27^{NS}	9.24**	7.45**	11.86**	14.22**	17.74**	19.28**	4.44*	14.07**	7.53**
F (C x N)	3.25*	3.36*	2.19^{NS}	8.44**	8.31**	1.26^{NS}	1.64^{NS}	3.30*	0.52^{NS}	0.64^{NS}	0.22^{NS}	8.98**
C.V. (%)	34.84	35.68	33.38	36.43	33.93	38.02	36.06	36.42	39.39	29.44	37.18	14.07

[1] For analysis, the data were transformed $(x + 0.5)^{1/2}$. Means followed by the same letter. in the column, were not statistically different at the 5% level (Tukey test). ^{NS} = not significant; * = significant at 5% probability; ** = significant at 1% probability.

to choose. Reductions were greater in the concentrations 5% and 10%, compared to the lower concentration and control, which indicates strong avoidance of the caterpillars of the extract.

Temporal development of the interaction between cultivars and neem extract doses was demonstrated, as there was a significant difference at 1, 3, 10, 15 and 120 minutes after caterpillar release (P≤0.01) (Table 2). At the 1- and 3-minute intervals, fewer caterpillars had visited cultivar IAC 503 treated with aqueous neem extract (both concentrations) than IAC 147 or the control (Table 2). The same trend was observed at 10, 15 and 120 minutes after caterpillar release (Table 2). Overall, appears that cultivar IAC 503 treated with 10% aqueous neem extract attracts caterpillars least. Other studies have revealed that the concentration of the extract and the time of exposure of the caterpillar to neem can influence mortality rate, Silva et al. (2015) comment that, although the insecticidal action of neem is known, there are no in-depth studies on post-ingestion insect behavior, the minimum concentration and the exposure period leading to lethality remain uncertain.

The interaction with leaf disc consumption was significant for *S. frugiperda* (P≤0,01) (Table 3). Cultivar IAC 503 leaf discs were consumed here less when treated with neem extract at both concentrations. Shannag *et al.* (2013) evaluating the effects of Azatrol (1.2 % azadiractina), Triple Action Neem Oil (70 % neem extract) and pure neem extract (100 % neem) for *Spodoptera eridania* (Cramer) (Lepidoptera: Noctuidae) reported that neem-based products did not completely inhibit food intake, but did reduce food intake considerably. These authors also noted that, while exposure to neem extract prolonged development duration, enhanced larval mortality and negative influenced in pupal ecdysis, intensity of effect depended on the product and dosage used.

There was a significant difference in larval mortality levels at 5 and 7 days after the start of the test, with caterpillars given peanut cultivar IAC 503 showing the highest mortality (P≤0.01) (Table 4). Of cultivars with erect growth habit, Campos & Boiça Junior (2012) found higher mortality among *S. frugiperda* larva fed on peanut cultivar IAC 22, and IAC Runner 886, among those with a creeping growth-form.

Table 2. Breakdown of the interaction between cultivars (*Arachis hypogaea*) versus doses (neem) of the average number of newly hatched *Spodoptera frugiperda* caterpillars attracted at 1, 3, 10, 15 and 120 minutes after release, by leaf disc treated or untreated with neem aqueous extract, in chance to choose tests under laboratory conditions. Temperature: 25 ± 1 °C; RH: 70 ± 10%; photophase: 14 h.

Cultivars (C)	Neem Extract (N)[1]			F (C)
	5%	10%	0	
		1 Minute		
IAC 503	0.27 Aa	0.20 Aa	1.00 Bb	5.79**
IAC 147	0.47 Aa	0.13 Aa	0.27 Aa	0.84^{NS}
F (N)	0.61^{NS}	0.09^{NS}	7.51**	
		3 Minutes		
IAC 503	0.27 Aa	0.13 Aa	1.07 Bb	7.03**
IAC 147	0.60 Aa	0.20 Aa	0.40 Aa	1.03^{NS}
F (N)	1.50^{NS}	0.08^{NS}	5.33*	
		10 Minutes		
IAC 503	0.13 Aa	0.13 Aa	1.53 Bb	17.04**
IAC 147	0.40 Aa	0.13 Aa	0.27 Aa	0.44^{NS}
F (N)	1.04^{NS}	0.00^{NS}	19.73**	
		15 Minutes		
IAC 503	0.20 Aa	0.20 Aa	1.53 Bb	8.31**
IAC 147	0.53 Aa	0.33 Aa	0.40 Aa	0.44^{NS}
F (N)	2.11^{NS}	0.34^{NS}	15.36**	
		120 Minutes		
IAC 503	0.27 Aa	0.07 Aa	1.60 Bb	14.70**
IAC 147	0.40 Aa	0.33 Aa	0.80 Aa	1.61^{NS}
F (N)	0.38^{NS}	0.97^{NS}	5.43*	

[1] For analysis, the data were transformed $(x + 0.5)^{1/2}$. Means followed by the same letter, in the column, were not statistically different at the 5% level (Tukey test). ^{NS} = not significant; * = significant at 5% probability; ** = significant at 1% probability.

Table 3. Breakdown of the interaction between cultivars (*Arachis hypogaea)* versus plant extracts (neem) for leaf disc dry mass consumed by *Spodoptera frugiperda* caterpillars, treated or not with natural products, in a chance to choose test under laboratory conditions. Temperature: 25 ± 1 ºC; RH: 70 ± 10%; photophase: 14 h.

Cultivars (C)	Neem Extract (N)[1]			F (C)
	5%	10%	Control	
IAC 503	0.20 Aa	0.18 Aa	0.65 Bb	16.55**
IAC 147	0.30 Aa	0.23 Aa	0.24 Aa	0.21^{NS}
F (N)	0.94^{NS}	0.33^{NS}	19.40**	

[1] Original data was transforme $(x + 0,5)^{1/2}$. Means followed by the same letter, in the column, were not statistically different at the 5% level (Tukey test). ^{NS} = not significant; * = significant at 5% probability; ** = significant at 1% probability.

Table 4. Larval, pupal and total mortality (larval + pupal) of *Spodoptera frugiperda* reared on peanut (*Arachis hypogaea*) cultivars treated with different doses of aqueous neem extract under laboratory conditions. Temperature: 25 ± 1 ºC; RH: 70 ± 10%; photophase: 14 h.

Cultivars (C)	Larval Mortality (%)[1]					Total Mortality (%)[1]
	after 1 day	after 3 days	after 5 days	After 7 days	after 10 days	
IAC 503	3.33 a	18.89 a	60.00 b	76.67 b	83.33 a	75.76 b
IAC 147	4.44 a	10.00 a	37.78 a	56.67 a	71.11 a	62.75 a
F (C)	0.15^{NS}	2.89^{NS}	9.25**	8.39**	3.86^{NS}	4.80*
Neem Extract (N)						
5%	3.33 a	18.33 b	60.00 b	86.67 b	96.67 b	96.67 b
10%	6.67 a	23.33 b	67.67 b	90.00 b	100.00 b	100.00 b
Control	1.67 a	1.67 a	20.00 a	23.33 a	35.00 a	25.93 a
F (N)	1.06^{NS}	6.55**	17.48**	63.50**	93.27**	578.21**
F (C x N)	1.06^{NS}	1.91^{NS}	0.08^{NS}	0.82^{NS}	1.96^{NS}	1.10^{NS}
C.V. (%)	128.13	138.76	86.69	63.70	49.35	53.06

[1] For analysis the data were arcsine transformed $(x/100)^{1/2}$. Means followed by the same letter, in the column, were not statistically different at the 5% level (Tukey test). [2] Dados insuficientes para análise estatística (variância nula). ^{NS} = not significant; * = significant at 5% probability; ** = significant at 1% probability.

Total mortality (caterpillars + pupae) differed between cultivars (P≤0.05), with *S. frugiperda* caterpillars fed with IAC 503 and IAC 147 showing mortality rates of 75.76 and 62.65%, respectively. Campos *et al*. (2011), evaluating resistance of upright and creeping growth habit peanut cultivars to *S. frugiperda*, found that cultivars IAC 503 and AC 147 were considered susceptible to pest attack, but there was no difference between them in terms of how strongly they were avoided, results contrary to those obtained in the present study. It is noteworthy that this studies also used the cultivars IAC 505, IAC 125, IAC Caiapó and IAC Runner 886. There was a significant difference in larval mortality levels for different doses of aqueous neem extract, at 3, 5, 7 and 10 days after the start of the test (Table 4), with the highest mortality larval being obtained when leaflets were treated with a 10% concentration, causing up to 100% caterpillar mortality by day 10. Total mortality (caterpillars + pupae) did not differ between the aqueous neem extract doses, with the 5% dose being the most recommended, since it caused 96.67% mortality, at half the dosage.

Viana & Prates (2003) reported 87.3% mortality in *S. frugiperda* caterpillars fed with corn leaves immersed in 1% aqueous neem extract, after 10 days of application. Roel *et al*. (2010), also working on *S. frugiperda*, showed that treatment using an artificial diet with neem oil at 0.4% concentration resulted in 100% mortality at the larval stage. The same authors also found that in the concentration of 0.05%, some caterpillars found it difficult to leave the exuvium, which remained attached to the body. In addition, histopathological changes were observed in the midgut region, where the peritrophic area showed changes such as degradation, thickening, fragmentation and subsequent loss of defense capacity against neem action by the epithelium. Correia *et al.* (2009) found that, for neem oil at concentrations of 0.5 and 1.0% there was a toxic effect, producing histolysis in the mesentery of *S. frugiperda* caterpillars, probably resulting in abnormal functioning of this tissue, altering enzymatic the secretion, nutrient absorption and cell renewal. The same authors also report that the extracts *Melia azedarach*, a plant in to the same family as *A. indica,* promoted in *S. frugiperda* the activation of the cytochrome P-450 system, an important mechanism for the degradation of toxic metabolites, thus indicating the presence of insecticidal action.

Maroneze & Galegos (2009) also found increased mortality during the development of *S. frugiperda* caterpillars treated with aqueous extract of *M. azedarach* during ecdysis, without being able to fully release the exuvium and head capsule. In the current study it was not possible to evaluate the effects of cultivars and doses of aqueous neem extract on pupal mortality due to an insufficient number of individuals (Table 4). Martinez & van Emdem (2001) reported for a wide range of insecticidal chemicals that, depending on the dose and the application mechanism, it may take some time for the active ingredient to act on the insect's metabolism, this can result in low mortality at the end of the larval stage, but high mortality in the pupal stage, indicating that the insecticidal effects of neem may involve several mechanisms.

Studies by Tavares *et al.* (2014) found that newly-hatched and first instar caterpillars are more susceptible to the toxic effects of the plant-derived toxins; one day-old eggs of *S. frugiperda* showed higher mortality than older eggs when exposed to neem extract, suggesting that more mature eggs were more resistant.

In the current study, there was no significant difference in larval mass between the tested peanut cultivars. Caterpillars reared on IAC 503 leaflets had an average weight of 0.0159 g, while in cultivar IAC 147, the observed value was 0.0140 g. However, weight of pupae differed significantly between cultivars (P≤0.05), so that pupae of caterpillars that had been raised on IAC 503 leaflets had greater mass than those raised on IAC 147 (0.1804 and 0.1533 g, respectively).

It was not possible to analyze the effect of doses of aqueous neem extract on caterpillar mass and pupae and adult longevity, since the natural product caused the mortality of all individuals during the larval period. Longevity of *S. frugiperda* adults, appeared not to differ between peanut cultivars. Quin-Gang & Xiao-Dong (2011) report that limonoids can alter

the growth regulation mechanisms, and affect fertility, of many insect species. Other studies have revealed the ability of AZ to affect cell division, thus causing apoptosis and cell degradation (Wang *et al.* 2015). Shu *et al.* (2017) reported that AZ can induce apoptosis in *S. frugiperda* Sf9 cells, helping to explain the insecticidal effect of this limonoid.

It stands out as the main results of the research that in choice tests newly-hatched *S. frugiperda* caterpillars were less attracted to the cultivar IAC 147 and consumed it in smaller volumes; for cultivar IAC 503 application of 10% neem aqueous extract reduces leaf consumption; the tested peanut cultivars did not affect the biological development of *S. frugiperda* and an 10% aqueous extract of neem causes 100% mortality of *S. frugiperda*.

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