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.
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 68-hydroxygedunin can be derived synthetically; while the limonoids nimbocinol, salannin and azadiradione can also be extracted from *A. indica* (DRIPHOUTH & MORGAN 2010; AYL-GUTIERREZ et al. 2018).

Neem extracts induce mortality by preventing insects from carrying out ecdysis (MORRUE & NISBIT 2000; GÖES et al. 2003). This occurs via induced mesenteric histolysis (CORREA 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 JÚNIOR 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 the FCAV/UNESP plant resistance laboratory, Jaboticabal-SP, at a temperature of 25 ± 1 °C, 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 JÚNIOR 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 °C 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)² and the percentage of mortality in arc sine (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 JÚNIOR 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) (Ps0.01) (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.
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 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 % azadirachtina), 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.

| Cultivars (C) | Minutes | Dry Mass CONSUMED (g) |
|--------------|---------|----------------------|
|              | 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              | 0.64 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              | 0.51 a              | 0.49 a              | 0.53 a              | 0.58 a              | 0.25 a              |
| F (C)        | 1.63cence 0.20 y 0.36 y 3.36 y 0.99 y 0.77 y 0.75 y 0.17 y 0.34 y 0.46 y 1.62 y 2.30 y
| 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*          | 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*          | 8.44**              | 8.31**              | 12.66*              | 16.44*              | 3.30*               | 0.52*               | 0.64*               | 0.22**              | 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.

### 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) | F (C) |
|--------------|------------------|-------|
|              | 5%               | 10%   |
|              | 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** |
| F (N)        | 0.61**           | 0.09** | 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** |
| F (N)        | 1.50**           | 0.08** | 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** |
| F (N)        | 1.04**           | 0.00** | 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** |
| F (N)        | 2.11**           | 0.34** | 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** |
| F (N)        | 0.38**           | 0.97** | 5.43** |
Total mortality (caterpillars + pupae) differed between cultivars (Ps0.05), 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 mesentry 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 ecysis, 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 EMDEN (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 (Ps0.05), 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 & XIAD-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). Shi 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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