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Determination of species composition and effective of plant extracts to prevent the eggs-lay of fruit flies, *Bactrocera* spp. infesting jackfruit

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Abstract
Determining the composition of fruit flies damage to jackfruit and effect of some plant extracts to prevent fruit fly egg laying was carried out from January to November 2022 at Southern Horticultural Research Institute (SOFRI) and the jackfruit farms at Tan Lap and Tan Thanh communes, Tan Thanh district, Long An province. The results of determining the composition of "Changai" jackfruit fruit fly species in 15 orchards revealed that there were two fruit fly species, *Bactrocera dorsalis* and *B. umbrosa* with a frequency of occurrence of 80.0% and 26.67%, respectively. The lab trial to study efficiency of five plant extracts *Allium sativum* (Garlic), *Allium cepa* (Onion), *Wedelia trilobata* (Wedelia), *Artemisia absinthium* (Mugwort), *Azadirachta indica* (Neem) to prevent egg laying of *B. dorsalis* was conducted; the results showed that two treatments of plant extracts *A. indica* and *W. trilobata* were highly effective against *B. dorsalis* with rates of 82.22% and 71.50% at 7 days after treatment. In laboratory conditions, 0.9% concentration neem seed extract was effective in preventing fruit fly oviposition on jackfruit fruits by 83.19%. In field conditions, the results of spraying neem seed extracts at a concentration of 0.9% with 8 times/fruit crop and 6 times/fruit crop (5 and 7 days/time, respectively) reduced the damage of fruit flies with the damage rate of 41.67% and 50.33%, respectively. The experimental results have an initial significance in contributing to the integrated pest management process of fruit fly damage on jackfruit.

Keywords: *Azadirachta indica,* fruit fly *Bactrocera dorsalis*, jackfruit, plant extract

Introduction
In Vietnam, jackfruit is considered a commercial fruit tree and is commonly grown in the Southern of Vietnam, particularly in the Mekong Delta provinces. The country's jackfruit growing area was 59,705 ha in 2020, up 2.9 times from 2017 (General Statistics Department, 2021) [4]. Fresh fruit products are promoted in order to expand export markets, with China currently considered the most important market. The export value of jackfruit reached over 105.4 million USD in the first six months of 2021, an increase of 42.4% over the same period last year. After dragon fruit, mango, and banana, it has the fourth highest export turnover (Saigon economy, 2021) [9]. Currently, the growing area of jackfruit is expanding, combined with the complicated climate change situation, leading to many difficulties in jackfruit production. In particular, pests are a major concern, fruit flies are considered the most dangerous and cause loss of yield on the current jackfruit orchards. The results of a survey from Southern Horticultural Research Institute reported that in Long An province, fruit flies damage up to 50% of jackfruit growing households, with the rate of damage in the garden being 20%. The commonly used method of controlling fruit flies by many farmers is intensive insecticide cover sprays. This is not only highly costly for the growers, but also damaging to the health of the farmer workers, the environment and non-target beneficial organisms (Ekesi & Billah 2007) [2]. Besides that, insecticide residues in agricultural products, especially fresh vegetables and fruits, increase the risk of cancer in consumers and producers (Fitriasari and Prijono 2009) [3]. As a result, it is necessary to find a safe and effective method to control the pest. Some plants contain many compounds, which have insecticidal, antifeedant, and growth inhibitor effect on this pest. Many compounds have no toxic effect or a low toxicity to non-target pests and humans, and are environmentally-friendly (Wei *et al.,* 2011) [16]. Plant products (like neem, garlic, and Melia in the present case) could be used as oviposition inhibitors in fruit flies.

Under field conditions, either spot spraying or traps containing plant extract-based food baits is required so that an appropriate strategy against fruit flies can be developed in the IPM module (Thakur and Gupta, 2012) [13]. Research results on some insecticidal extracts were found to be effective on harmful insects such as neem extract (*Azadirachta indica*) with active ingredient Azadirachtin capable of preventing *Thrips*, brown planthoppers (*Nilaparvata lugens*) and silkworms (*Bombyx mori*) (Tuan, 2002) [15]. Neem is known to affect more than 200 species of insects, Arrests pupae development, retards growth, toxic to larvae of Oriental fruit fly. Neem leaves (*Azadirachta indica*) inhibited oviposition of medflies *Ceratitis capitata* at a concentration of 18,000 ppm (Silva, *et al*., 2012) [11]. Neem extract controlled females from laying eggs *Bactrocera* spp. on tomatoes and fruits (Thakur and Gupta, 2013) [14]. *Wedelia trilobata* L. was an essential oil plant species with highly bioactive components, the most important of which is α-pinene (34.96%) (Li *et al*., 2012; Khater *et al*., 2015; Satongrod *et al*., 2021) [7, 6, 10]. Biological pest control measures, particularly for fruit flies that cause jackfruit damage, are required to produce safe jackfruit products that do not contain pesticide residues. To help reduce fruit fly populations in the garden, it is necessary to understand the composition of fruit fly species on jackfruit orchards and to research herbal extracts capable of preventing egg laying.

Materials and Methods

Time: From January to November 2022.

Place: The studies on determining the species composition of fruit flies damaged to jackfruit and effect of some plant extracts on jackfruit were conducted at SOFRI and the jackfruit farms at Tan Lap and Tan Thanh communes, Tan Thanh district, Long An province.

Materials

Fruit flies, jackfruit farms 5 years; Magnifier, Olympus fluorescence microscope, Soxhlet distillation, sprayer; Soft brush, vial, blotting-paper, plastic bag, petri dish, slide, lame, scissors, cage netting (2 x 2 x 2 m), traps, sawdust; Alcohol 98%, metanol, distilled water; Plant extracts: *Azadirachta indica*-neem seeds, *Allium sativum*-bulb of garlic, *Allium cepa*-bulb of onion, *Wedelia chinensis*-stems and leaves, *Artemisia vulgaris*-stems and leaves of mugwort.

Methods

Experiment 1: Determining the species composition of fruit flies damaged to jackfruit

Fruit samples damaged by fruit flies on "Changai" jackfruit orchards in the fruit-bearing stage were collected from 15 orchards at Tan Lap and Tan Thanh communes, Tan Thanh district, Long An province, each orchard collecting from 3-5 fruits with harmful fruit fly symptoms (Fig. 1). Fruit samples were collected and brought to the Plant Protection Laboratory of SOFRI.

Each jackfruit was placed on a plastic tray with a 5 cm thick layer of sawdust and placed inside each net cage at room temperature to pupate into larvae. The pupae were placed in net cages until they turned into adults to identify the species according to the fruit fly identification document (Plant Health-Australia, 2011) [8]. Fruit fly species composition and abundance of fruit fly population in the field prevalence of each species was recorded. Appearance frequency of fruit flies followed the formula:

Frequency (%) = (Number of species present/ Total number of observed orchards) x 100; Popularity is divided into 4 levels: + (frequency <5%); ++ (frequency 5-<25%); +++ (frequency 25-<50%); ++++ (frequency ≥50%).

Experiment 2: Efficiency of some plant extracts to prevent fruit fly *Bactrocera dorsalis* from laying eggs on jackfruit

Preparing plant extracts: *Azadirachta indica*-neem seeds, *Allium sativum*-bulb of garlic, *Allium cepa*-bulb of onion, *Wedelia chinensis*-stems and leaves, *Artemisia vulgaris*-common mugwort stems and leaves were extracted using a Soxhlet distillation (Behr Labor Technik-R 106S) according to Dodia *et al.* (2008) [1].

Preparation of fruit flies: For the experiment, choose the *B. dorsalis* species with the highest frequency on jackfruit orchards. Collecting jackfruit fruits with fruit fly damage to the net cage; the pupae develop into larvae after 15-20 days. When the adult larvae are 10 days old, collect the larvae and place them in a separate net cage (adding water and sugar as food for the larvae).

The trial was arranged in a randomized complete design (RCD) consisting of six treatments (five treatments at five plant extracts were *Allium sativum* (bulb of garlic), *Allium cepa* (bulb of onion), *Wedelia trilobata* (stems and leaves of wedelia), *Artemisia absinthium* (stems and leaves of mugwort), *Azadirachta indica* (neem seeds), and control with water, respectively) with four replications for each treatment with a net cage (30 x 30 cm in size) per replication. A net cage was put into ten fruit fly adults of the same age (5 adult males and 5 adult females). Select jackfruits at 95 days after the fruit set, wash them, cut them into 10 x 10 cm pieces, and soaked into plant extracts at concentration of 0.5% for 30 seconds then put them in each net cage. Use a magnifying glass to count the number of eggs laid on the jackfruit pieces every 24 hours (Proceed to replace with new jackfruit pieces made in the same manner as the original). All net cages were kept at 28 ± 1ºC and 65 ± 5% RH in lab conditions. The number of eggs in each treatment at 1, 2, 3, 4, 5, 6 và 7 days after treatment was recorded. The efficiency of deterrence of oviposition (H%) of plant extracts was calculated according to the formula (Thakur và Gupta, 2013) [14]

H (%) = [(C-T)/C] x 100 (C: Number of eggs laid on the control; T: Number of eggs laid on the treatment).

Experiment 3: Efficiency of different concentrations of neem seed extracts of inhibiting the oviposition of fruit fly *Bactrocera dorsalis* on jackfruit in the laboratory conditions: The results of experiment 2 revealed that neem seed extract was effective in preventing the fruit fly *B. dorsalis* from oviposition on jackfruit. Under laboratory conditions, conduct experiments to determine the optimal concentration of neem seed extract to effectively control the oviposition of fruit fly larvae.

The trial was arranged in a RCD consisting of six treatments (five treatments at five concentrations of neem seed extract at 0.1%, 0.3%, 0.5%, 0.7%, 0.9%, and control treatment which was soaked with water, respectively) with four replications for each treatment with a net cage per replication. A net cage was put into ten fruit fly adults of the same age (5 adult males and 5 adult females). Select jackfruits at 95 days after the fruit set, wash them, cut them into 10 x 10 cm pieces, and soaked into plant extracts for 30 seconds then put them in each net cage. Use a magnifying glass to count the number of eggs laid on the jackfruit pieces every 24 hours (Proceed to replace

with new jackfruit pieces made in the same manner as the original). All net cages were kept at 28 ± 1 ºC and 65 ± 5% RH in lab conditions. The number of eggs in each treatment at 1, 2, 3, 4, 5, 6 và 7 days after treatment was recorded. The efficiency of deterrence of oviposition (H%) of neem seed extract at different concentraions was calculated according to the formula (Thakur và Gupta, 2013) [14].

Experiment 4: Efficiency of neem seed extract against fruit fly *Bactrocera dorsalis* on jackfruit in field conditions

The experiment was performed at the jackfruit farms 5 years at Tan Lap communes, Tan Thanh district, Long An province to evaluate the efficacy of neem seed extract and which concentration showed high effectiveness to prevent fruit fly laying eggs in lab conditions. The trial was arranged in a randomized complete blocks design (RCBD) consisting of six treatments (Table 1) with four replications per treatment with a jackfruit tree at 45 days old fruit stage (selected jackfruit trees bearing 3-5 fruits). Neem seed extract at 0.9% concentration was applied by a motor sprayer to cover the canopy and the fruit of the tree. The volume of the applied mixture was 600 liters per ha. Infested rate of fruit at harvest time was recorded.

Infected rate (%) = Number of infested fruits/Total number of fruits observed.

Table 1: Experimental treatments

Treatment	Time interval (number of days)	The number of sprayings (times)
T1	5	8
T2	7	6
T3	10	4
T4	14	3
T5	21	2
T6	Control	Control

Data analysis

Data was collected and analyzed using analysis of variance (ANOVA), and Duncan's Multiple Range Test (DMRT) was used for means comparison when treatments were significant using MSTATC program.

Results and Discussions

The species composition of fruit flies damaged to jackfruit

The composition of fruit fly species damaged to 15 "Changai" jackfruit orchards in Tan Lap and Tan Thanh communes, Tan Thanh district, Long An province revealed two species that appeared to cause damage to "Changai" jackfruit trees (Fig. 2). *Bactrocera dorsalis* accounted for 62.34% and *Bactrocera umbrosa* accounted for 37.66%, with frequencies of 80.0% (very popular) and 26.67% (popular), respectively (Table 2).

Table 2: The species composition, frequency and popularity of fruit flies damaged to jackfruit

The species composition	Percentage of species composition (%)	Appearance frequency (%)	Popularity
Bactrocera dorsalis	62.34	80.00	++++
Bactrocera umbrosa	37.66	26.67	+++

Popular (Appearance frequency 5-<25%); (++++) Verry popular (Appearance frequency ≥ 50%).

The efficiency of some plant extracts to prevent fruit fly *Bactrocera dorsalis* from laying eggs on jackfruit

The results showed that efficiency preventing to fruit fly *B. dorsalis* laying eggs had a statistically significant difference at 7 days after treatment (Table 3). The *A. indica* (neem seed) treatment prevented fruit flies from laying eggs at a rate of 82.22%, which was not statistically significant difference when compared to the *W. trilobata* (Wedelia) treatment (71.50%) but was very significant when compared to the *Allium cepa* (onion), *Artemisia absinthium* (mugwort), and *Allium sativum* (garlic) extract treatments (67.86%, 63.28%, and 42.82%), respectively. The extract of neem *A. indica* with dichloromethane (888 ppm) treated the fruit fly *Ceratitis capitata* significantly reduced the number of eggs laid by 80% and the hatching rate by 30% at 8 day after treatment. The author indicated that the Neem extracted with dichloromethane affected the reproduction of *C. capitate* (Silva *et al*., 2013) [12].

Table 3: The percentage of plant extracts prevent fruit flies oviposition of *Bactrocera dorsalis*

Treatment	Percent deterrence of oviposition (%)						
	1 DAT	2 DAT	3 DAT	4 DAT	5 DAT	6 DAT	7 DAT
Allium sativum	31.06^{d}	22.84^{c}	23.48^{c}	10.56^{d}	23.88^{d}	41.81^{c}	42.82^{c}
Allium cepa	47.38^{c}	42.42^{b}	54.89^{ab}	41.45^{b}	49.40^{bc}	52.40^{bc}	67.86^{b}
Wedelia trilobata	66,55^{b}	49,75^{b}	44.55^{b}	59.18^{a}	54.50^{b}	64.46^{b}	71.50^{ab}
Artemisia absinthium	36.50^{d}	38.96^{bc}	41.47^{b}	20.34^{c}	31.43^{c}	47.71^{c}	63.28b^{c}
Azadirachta indica	89.78^{a}	73,48^{a}	56.22^{a}	60.38^{a}	70.78^{a}	79.40^{a}	82.22^{a}
CV (%)	8.6	18.4	21.43	18.24	8.21	15.79	22.49
F	**	**	**	**	**	**	**

In a column, means followed by same letters are not significantly different at 1% probability level by Duncan's Multiple Range Test (DMRT), DAT = Day after treatment.

The efficiency of different concentrations of neem seed extracts of inhibiting the oviposition of fruit fly *Bactrocera dorsalis* on jackfruit in the laboratory conditions: At 7 days after treatment, neem seed extract at a concentration of 0.9% was effective in preventing laying eggs of fruit flies *B. dorsalis* on jackfruit (83.18%) with a statistically significant difference when compared with the other treatments. Next was the concentration of 0.7% reaching 52.31% (Table 4) (Fig. 3).

Table 4: The percentage of fruit fly oviposition inhibited of *Azadirachta indica* extract at different concentrations

Treatment (Neem seed extract)	Percent deterrence of oviposition (%)						
	1 DAT	2 DAT	3 DAT	4 DAT	5 DAT	6 DAT	7 DAT
0.1%	33.76^{d}	23.58^{e}	16.41^{d}	30.07^{d}	26.71^{c}	16.30^{d}	15.33^{d}
0.3%	37.84^{d}	35.83^{d}	29.61^{c}	47.94^{c}	34.81^{bc}	33.35^{c}	23.31^{cd}
0.5%	48.28^{c}	43.14^{c}	36.56^{bc}	56.46^{b}	40.28^{b}	49.03^{b}	36.83^{c}
0.7%	63.28^{b}	54.22^{b}	45.10^{b}	60.44^{b}	53.96^{ab}	59.38^{ab}	52.31^{b}
0.9%	75.14^{a}	92.13^{a}	75.17^{a}	65.02^{a}	57.58^{a}	66.50^{a}	83.19^{a}
CV (%)	11.83	9.33	24.42	8.25	13.72	19.41	11.95
F	**	**	**	**	**	**	**

In a column, means followed by same letters are not significantly different at 1% probability level by Duncan's Multiple Range Test (DMRT), DAT = Day after treatment.

The efficiency of neem seed extract against fruit fly *Bactrocera dorsalis* on jackfruit in field conditions

The treatment of neem seed extract (T1, T2) at a concentration of 0.9% with 8 times spraying (5 days/time) and 6 times spraying (7 days/time) had a low rate of fruit fly damage of 41.67% and 50.33%, respectively. There was a statistically significant difference compared with the control T6 (91.67%) and the treatments T5, and T4 with the rate of fruit fly damage were 75%, 75%, and 66.67%, respectively. The result found that spraying neem seed extract at a concentration of 0.9% with the number of sprays 6-8 times/crop was effective in managing jackfruit fruit flies.

Table 5: The percentage of infested jackfruit fruit (%) of fruit fly on in field condition

Treatment	Number of spraying	Percentage of infested (%)
T1	8 times (5 days/time interval)	41.67^{d}
T2	6 times (7 days/time interval)	50.33^{cd}
T3	4 times (10 days/time interval)	66.67^{b}
T4	3 times (14 days/time interval)	75.00^{ab}
T5	2 times (21 days/time interval)	75.00^{ab}
T6	Control	91.67^{a}
CV (%)		30.12
F		**

In a column, means followed by same letters are not significantly different at 1% probability level by Duncan's Multiple Range Test (DMRT).

Fig 1: Fruit fly symptoms on the fruit appeared opaque white resin, brown rot and soft spots (from left to right)

Fig 2: A) Pupae and adult of *Bactrocera umbrosa*; B) Pupae and adult of *Bactrocera dorsalis*

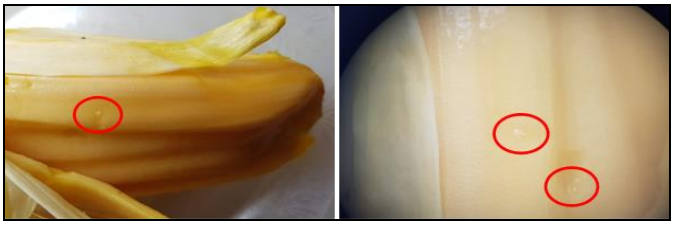

Fig 3: Eggs laid scattered by fruit fly *Bactrocera dorsalis* on the jackfruit fruits

Conclusion

The results of determining the composition of the dangerous fruit fly species on the "Changai" jackfruit orchard revealed two species, *Bactrocera dorsalis* and *B. umbrosa* with occurrence rates of 80.0% and 26.67%, respectively. Under laboratory conditions, the Neem seed extract was most effective in controlling the egg-laying ability of fruit fly *B. dorsalis*, with the highest egg-laying rate of 82.22%, and the Neem seed extract at a concentration of 0.9% was most

effective in preventing *B. dorsalis* from laying eggs at 83.19%. Spraying Neem seed extract 8 times/fruit crop (5 days/time) and 6 times/fruit crop (7 days/time) effectively reduced fruit fly damage on jackfruit trees with a high rate of success. The relative damage rates were 41.67% and 50.33%.

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