REPELLENT ACTION OF CASHEW (*Anacardium occidentale* L.) NUT SHELL LIQUID (CNSL) ON ADULT PAPAYA FRUIT FLY *Bactrocera dorsalis* (HENDEL) (DIPTERA: TEPHRITIDAE)

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ABSTRACT

The fruit fly, *Bactrocera dorsalis* is a major pest of papaya and known to cause economic losses to the agricultural industry globally. It attacks the fruits causing premature ripening and rotting. This study aims to investigate the repellency activity of the liquid extract of the cashew nut shell using hexane and dichloromethane as extracting solvents. The repellent action of the extracts was tested by dissolving 2, 4, 6, 8, 10 µL of the extracts in 1 ml of acetone to produce concentrations of 0.07, 0.14, 0.21, 0.28, and 0.35 µL/cm^2. An 85mm filter paper was cut into two equal halves which penetare with treatment and control. Both equal half discs were air-dried to evaporate the solvent completely. The treated halves to untreated halves were then remade by attaching them into full discs. The treated filter paper was placed in a petri dish, and ten fruit flies were released at the centre of the petri dish and then covered. Repellency effect was observed after 12 and 24hrs and repeated for ten replicates. The hexane extract at a concentration of 0.35 µL/cm^2 gave a higher repellency of 76% as compared to other concentrations tested while the dichloromethane extract recorded 48% repellency at the same concentration after 24 hours. Thus, the hexane extract showed a significantly higher repellency effect on fruit flies as compared to dichloromethane at 0.35 µL/cm^2. It could be concluded that hexane is a better candidate to be used in an integrated pest management program, and it could possibly reduce the problem of pesticide residues in the environment.

Keywords: *Bactrocera dorsalis*, cashew shell nut liquid, dichloromethane extract, fruit fly, hexane extract, repellency

ABSTRAK

Lalat buah, *Bactrocera dorsalis* merupakan perosak utama betik dan boleh menyebabkan kehilangan ekonomi terhadap industri pertanian di peringkat global. Ia menyerang dan menyebabkan buah masak secara tidak matang dan membusuk. Kajian ini bertujuan untuk mengkaji aktiviti penghalau bagi ekstrak cecair kulit luar gajus menggunakan heksana dan diklorometana sebagai pelarut ekstrak. Tindakan penghalau ekstrak diuji dengan melarutkan 2, 4, 6, 8, 10 µL ekstrak ke dalam 1 ml aseton untuk menghasilkan kepekatan 0.07, 0.14, 0.21, 0.28, and 0.35 µL/cm^2. Kertas turas 85mm dipotong kepada dua bahagian. Separuh kertas turas telah dirawat dengan aseton sahaja sebagai kawalan. Rawatan kimia dan dan kawalan pada separuh kertas turas dikeringkan untuk peruwapan pelarut sepenuhnya. Keseluruhan kertas turas dicantumkan dengan melekatkan separuh rawatan kepada separuh kawalan pada dimensi yang sama. Kertas turas rawatan ditempatkan di dalam piring Petri dan sepuluh ekor lalat buah dilepaskan di tengah piring Petri dan kemudiannya ditutup. Kesan penghalau diperhatikan selepas 12 dan 24 jam dan diulang sebanyak sepuluh replikasi. Ekstrak heksana pada kepekatan 0.35 µL/cm^2 telah memberikan kesan penghalauan yang tinggi pada 76% dibandingkan dengan kepekatan lain yang diuji, sementara ekstrak dikloromethana mencatatkan 48% pengahalauan pada kepekatan yang sama selepas 24 jam. Dengan demikian, ekstrak heksana menunjukkan kesan penghalauan yang tinggi secara signifikan terhadap lalat buat berbanding dikloromethana pada 0.35 µL/cm^2. Dapat disimpulkan heksana merupakan calon terbaik untuk digunakan di dalam program pengurusan perosak bersepadu dan berkemungkinan mampu mengurangkan masalah sisa pestisid dalam persekitaran.

Kata kunci: *Bactrocera dorsalis*, cecair kulit luar gajus, ekstrak dikloromethana, ekstrak heksana, lalat buah, penghalau

INTRODUCTION

Insect reaction to external stimuli in the form of avoidance behaviour has been used as a tool in insect pest management and control for centuries (Debboun & Moore 2006). As a result, insects' behaviour to external stimuli has been a welcome strategy to many who were concerned with insecticide residue in food crops. The *Bactrocera dorsalis* (Hendel) species complex is a group of closely related species of true fruit flies. They include the Asian papaya fruit fly, *B. papayae* Drew & Hancock, the Philippine fruit fly, *B. philippinensis* Drew & Hancock (Schutze et al. 2015) which are amongst the world's most important pests of papaya (Khamis et al. 2012).

Botanical insecticides had been in use for thousands of years. They have been reported to possess contact toxicity (Asmanizar et al. 2019), systemic or repellent action against their target pests (Rajashekar et al. 2012). However, decades ago, the introduction and use of synthetic insecticides, which began in the 1940s, led to the neglect of botanical insecticides owing to their high efficiency, fast action, ease of use and low cost. However, about twenty years after introducing synthetic insecticides, several adverse side effects associated with their use that were not initially thought of at the time of their introduction began to surface. These include the development of insect resistance, pesticide food contamination, environmental pollution problems, the disruption of natural balance, toxicity to non-target organisms, and most importantly, negative impact on human health (Grdisa & Grsic 2013).

For many years, the production and application of insect repellent compounds were directed towards public health concerns such as mosquitoes' control. Despite this long history of their existence, only four insect repellents were in use before World War II. These were oil of citronella, sometimes used as hairdressing for head lice, dimethyl phthalate discovered in 1929; (Indalone®), which was later patented in 1937 as Rutgers 612, which became available in 1939 (Katz et al. 2008; Peterson & Coats 2001). At the onset of World War II, a new formulation for use by the military known as 6-2-2; (six parts dimethyl phthalate, two parts Indalone and two parts Rutgers) was introduced. This new formulation however, failed to provide the desired protection for military personnel deployed around the world. In 1953, the insect repellent properties of N, N-diethyl-meta-toluamide (DEET) were discovered. The first DEET product was introduced in 1956, and since then, DEET has reigned as the most effective and broadly used insect repellent for the last six decades. DEET has been found to have a strong safety record, and it provides excellent protection against ticks, mosquitoes, and other arthropods (Katz et al. 2008). However, in recent years toxic effects associated with the use of this product have been reported. These include encephalopathy in children, hypotension and decreased heart rate (Lupi et al. 2013; Peterson & Coats 2001).

In view of these adverse side effects associated with synthetic insecticides, interest began to grow again into the investigation of various alternative measures different from the application of synthetic insecticides. These include management practices that focused on developing and using natural plant protection agents such as plant-derived insecticides (Grdisa & Grsic 2013).

Plant-derived insecticides, otherwise referred to as botanical insecticides like their counterpart; the synthetic insecticides act on their targets in many ways. One of which is their repellent activity due to the presence of secondary metabolites (Chaudhary et al. 2017). For thousands of years, man has exploited this repellent and fumigating action of plants against insects. The simplest way by hanging or burning plants in homes to drive away nuisance mosquitoes. Later as oil formulations applied to the skin or clothes, whereby it repels the insects. This practice is still widely used in developing countries (Debboun & Moore 2006; Debboun & Strickman 2013).

The repellent action of plants and plant products against pests has been reported by Khater (2012) and Maia & Moore (2011) which showed their roles as defensive phytochemicals (Isman & Akhtar 2007; Mann & Kaufman 2012). The beauty about these compounds is that they are often easily decomposed by various common microbes in most soils. Consequently, the potential for environmental contamination is reduced (Khater 2012). These compounds are easily decomposed by UV light from the sun, making them disappear from the environment in a few days after their application in the field (Miresmailli & Isman 2006). However, the short residual activity would necessitate the frequent and repeated application of the insecticide to ensure the maintenance of a low pest population (Showler 2017).

Repellent action against *Bactrocera dorsalis* is a highly desired alternative to manage the pest. This approach will ensure that the papaya fruits will have less contact with chemicals that may, in most cases, enters the food chain and end up reaching the consumer. Papaya fruits are highly prone to attacks by the pest, and any protective treatment by way of chemical sprays could cause residual toxicity. This phenomenon is utilized to protect crops and, by extension addressing the risk associated with the direct consumption of residues derived from the direct use of pesticides. In most cases, the papaya fruit does not undergo any form of processing after harvest compared with many field crops. It is consumed in the raw form, thus, exposing consumers to pesticide residues that remained in the fruit after harvest. Thus, repelling the pest from the fruits will cause fewer health hazard to the consumer.

Several studies by different authors (Ilyas et al. 2017; Rehman et al. 2009) have been reported on the repellent activity of botanical extracts against different *Bactrocera* species. These include neem (*Azadirachta indica* and turmeric *Curcuma longa* L). However, not many researches have far been reported on the repellent action of cashew nut shell liquid (CNSL) against *B. dorsalis*, a major pest of papaya. Recognizing the fact that the papaya fruits are most of the time consumed directly from the farm without any form of pesticide decontamination, therefore any management strategy that will limit the use of insecticides is needed to reduce pesticide poisoning.

The objective of this study was to evaluate the repellent action of CNSL using hexane and dichloromethane as extracting solvents against *B. dorsalis* under laboratory conditions through the behavioural reaction of avoidance. It is envisaged that the results obtained from this study will be another alternative in the management of a major pest of papaya.

MATERIALS AND METHODS

Soxhlet Extraction

Natural products such as plants extract to provide an enormous opportunity for new compound discoveries (Cos et al. 2006). Soxhlet extractor, developed by von Soxhlet (Luque de Castro & Priego-Capote 2010) was used to extract the CNSL from the nuts using two different solvents hexane and dichloromethane. In each of the extraction procedures, 40 grams of the smoothly ground CNS powder was put in a paper thimble (Favorit cellulose extraction thimbles: size 43 x 123 mm) and mounted on the Soxhlet apparatus. The heating mantle was set to the appropriate boiling point of the different solvents; 68°C for hexane and 39.6°C for dichloromethane. For each procedure, two litres of solvent were used. The apparatus was allowed to run for 3h until the color of the solvent in the Soxhlet chamber became clear. The CNSL extract was concentrated using a Buchi 461 water bath with a Buchi 011 Rotavapor set at 100 rpm to remove the excess solvent. The temperature of the water was set to the boiling point of the different solvents (68°C for hexane and 39.6°C for dichloromethane). The CNSL extract, dark brown in colour was allowed to run in the Rotavapor for 1h before being placed in an Ecocell oven set at 40°C and dried to constant weight. The extract was then transferred into a vile and used as a stock solution from which different concentrations were prepared.

Repellency Test

The petri dish bioassay method which allows test insects to choose their area preference within the petri dish described by McDonald et al. (1970); Zapata & Smagghe (2010) and used by (Benelli et al. 2013; Dorla et al. 2017; Siskos et al. 2009) for contact toxicity bioassay against some *Bactrocera* species was used. The method was modified and adapted for the repellency test in this study to evaluate the repellent action of CNSL extracts against *B. dorsalis*. Plastic Petri dishes of the following dimensions were used: bottom, internal diameter 8.5 cm; rim height, 1.3 cm; and lid, internal diameter 9.0 cm. A 2cm x 2cm opening was made on the top lid, and a piece of mosquito netting was glued over the entire opening for ventilation. Test areas consisted of an 8.5 cm diameter Whatman No.1 filter paper which was cut into two halves with each area of 28.36 cm^2. Solutions were prepared by dissolving 2, 4, 6, 8 or 10 µL of each CNSL extract in 1mL acetone to produce concentrations of 0.07, 0.14, 0.21, 0.28, and 0.35 $\mu L/cm^2$ of extract. These extracts were then stirred using an electric stirrer (Smith Votex Mixer MX-5) for a minute. One millilitre of each extract was applied to a half filter paper disc as uniformly as possible with a pipette and allowed to dry for 1h at room temperature. The other half part of these filters paper were treated with acetone alone which served as the control. All the extract-treated and control filter paper discs were air-dried to evaporate the solvent completely. Full discs were then remade by attaching the extract-treated half discs to the untreated (control) half discs of the same dimensions with the sellotape. Each filter paper was placed in a Petri dish and because of the size of the test insects, the number of insects per Petri dish was limited to ten. Thus, ten adult male flies that were initially immobilized by chilling them a minute. They were then transferred individually at the centre of each filter paper disc in the Petri dishes and then covered with the lid. The layout of the experiment was a Completely Randomized Design

(CRD). Each treatment was replicated ten times so that 100 insects were assayed per concentration, making a total of 500 insects for the whole experiment. The number of insects present on control (Nc and treated (N_T) strips were recorded after 12 and 24 h exposure.

Statistical Analysis

Percentage repellency (PR) values were calculated as PR = $[(N_C-N_T)/(N_C+N_T)] \times 100$ (where N=number of insects, C=control, and T=treatment). The percentage of repellency (PR) values were analyzed using ANOVA after arcsin-transforming them (Arcsin √ x/100) (Buxton et al. 2017). ANOVA was performed with the concentration being the factor. This was followed by Tukey's multiple comparison test to examine if the means were significantly different using SPSS Software Version 24. Mean (± SE) of untransformed data are reported. An independent t-test was done on the mean percentage repellency for the hexane and dichloromethane extracts after 24h time interval to determine if there was any significant difference between the extracts.

RESULTS

The repellent activity of hexane and dichloromethane extracts of CNSL against the papaya fruit fly *B. dorsalis* at different concentrations for 12 and 24 hours are demonstrated in Table 1 and 2. Results showed that both the hexane and dichloromethane extracts manifested repellent activity against *B. dorsalis*. The PR values for the extracts was significantly influenced by the concentration applied. Maximum repellent action was recorded at the highest concentration of 0.35µL/cm^2 after 24 hours of exposure for both the hexane and dichloromethane extracts.

For the hexane extracts, there was a significant difference between the treatments after 12h of exposure ($F=16.102$; df 4,45; $p<0.05$) (Table 1). The PR values after 12 hours of exposure for the hexane extracts applied at concentrations ranging from 0.07- 0.35µL/cm^2 were between 40% – 62%. For the 0.35µL/cm^2 concentration, the PR value was 1.6 folds higher than the PR value of the 0.07 µL/cm^2 concentration ($P<0.05$).

Table 1. Repellent activity of hexane extracts of CNSL against the papaya fruit fly *B. dorsalis* exposed to different concentrations at 12 and 24 hours. PR represents percentage repellency

Concentration µL/cm^2	**Mean PR (%) ± SE**	**Mean PR (%) ± SE**
	12 h	**24 h**
0.07	40.0±0.0a	60.0±0.0a
0.14	40.0±0.0a	60.0±0.0a
0.21	42.0±2.0ab	60.0±0.0a
0.28	52.0±4.4c	64.0±2.7b
0.35	62.0±3.6d	76.0±2.7c

*Means within the same column, followed by the same letter are not significantly different.

Similarly, the PR values after 24h exposure of hexane extracts also showed a statistical difference between the treatments ($F=39.5$; df 4,45; $p<0.05$). The PR values ranged from 60% to 76% for 0.07 and 0.35µL/cm^2 concentrations, respectively recording a 1.2 fold increase in repellency. This was found to be statistically significant ($P<0.05$). In addition, the PR value for 0.21µL/cm^2 concentration was also significantly different from the PR value for 0.28 µL/cm^2 ($p<0.05$).

The PR values for dichloromethane extracts after 12h exposure showed a 2.0 folds increase between the lowest concentration of 0.07µL/cm^2 and the highest concentration of 0.35µL/cm^2 (Table 2). The highest concentration of (0.35µL/cm^2) was statistically different in PR values from all the other treatments ($P<0.05$) except for the 0.28 µL/cm^2 concentration ($P>0.05$). The lowest concentration of 0.07µL/cm^2 was also lower in PR values from all the other treatments. However, it was observed that the treatments were statistically different after 12 hours of exposure (F=31.001; df 4,45; p=0.05).

There was a statistical difference between the treatments after 24h of exposure for the dichloromethane extracts (F=19.4; df 4,45; $p<0.05$). It was observed that the PR values of the lowest concentration of 0.07 µL/cm^2 showed an increase of 1.9 folds after 24 hours exposure. As for the highest concentration of 0.35µL/cm^2, the PR value increased by 1.09 folds after 24 hours time exposure. However, within the 24 hours exposure, the increase in PR values between the lowest and the highest concentration was only 1.14 folds (Table 2).

Table 2. Repellent activity of dichloromethane extracts of CNSL against the papaya fruit fly *B. dorsalis* exposed to different concentrations at 12 and 24 hours. PR represents percentage repellency

Concentration µL/cm^2	**Mean PR (%) ±SE**	**Mean PR (%) ±SE**
	12h	**24h**
0.07	22.0±2.0 a	42.0±2.0a
0.14	24.0±2.7ab	42.0±3.6a
0.21	36.0±6.5c	46.0±4.3ab
0.28	42.0±7.4cd	48.0±4.0bc
0.35	44.0±7.7d	48.0±4.4bc

*Means within the same column, followed by the same letter are not significantly different.

An independent t-test to compare the repellent action of CNSL against *B. dorsalis* between the hexane and dichloromethane extracts after 24h exposure indicated that hexane extracts gave a higher repellency. The mean percentage repellency observed was 44.52% for hexane extracts compared to 36.6% for dichloromethane extracts (Figure 1). These figures were significantly different to one another when analyzed using independent T-test (T=3.41; $p<0.05$).

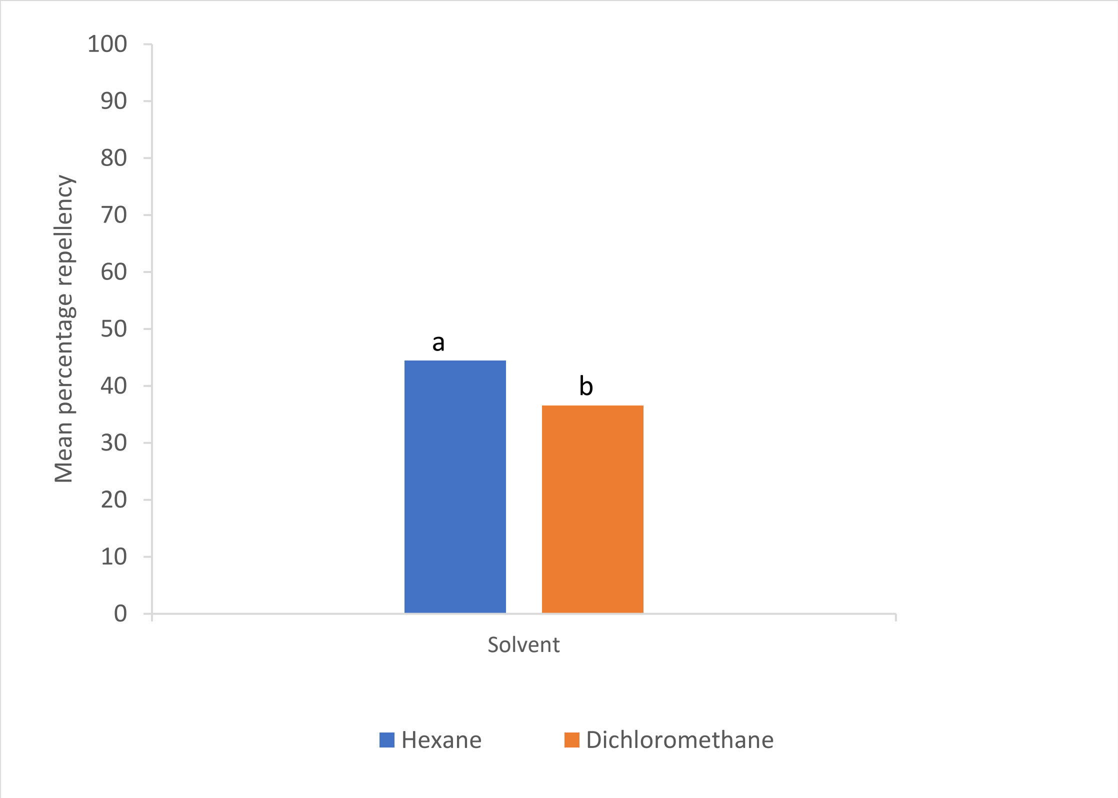

Figure 1. Mean percentage of repellency after 24h of exposure of *B. dorsalis* on hexane and dichloromethane extracts. Different alphabet indicates no significant differences using independent T-test

DISCUSSION

The results from this study showed that the repellent action of the hexane extracts was higher than the dichloromethane extracts after 24 hours exposure. The higher boiling point of hexane compared to the dichloromethane may cause the hexane extracts to be more stable and not easily evaporated. In which therefore, allowed these hexane extracts to possess higher repellent activity than the dichloromethane extracts.

The potential of CNSL extract as a repellent against *B. dorsalis* has been demonstrated in this study. Direct application of synthetic insecticides on the papaya fruits, which are normally consumed directly from the farm could now be avoided to reduce the risk of pesticide poisoning among consumers. Furthermore, the repellent action of the extracts would prevent the female insects from laying their eggs inside fruits. Contact insecticides which normally used to control adult pests, remain on the surfaces of treated fruits. They, therefore, are unable to penetrate the fruits and killed the eggs or developing larvae.

A study by Silveira et al. (2019) on the repellent effect of CNSL showed that bamboo sticks treated with CNSL repelled the bamboo borer *Bambusa vulgaris* from penetrating them. Similarly, CNS extract was found to be highly toxic to 1st, 2nd and 3rd nymphs of *Bemisia tabaci* Genn (Andayanie & Ermawati 2019).

In this study, the 76% repellency after 24 hours of the exposure recorded for the hexane extract was quite remarkable. Repelling this number of pests in any given population could provide a high degree of protection, especially when used in combination with other pest management strategies such as sanitation. The findings from this study match other investigations on the repellent action of plant extract other than CNSL against *Bactrocera spp* (Khan et al. 2016; Jaleel et al. 2020). For example, (Hossain & Khalequzzaman 2018) reported a strong repellency against *Bactrocera cucurbitae* (Coquillett) when it was exposed to the extracts of *Azadirachta indica* A. Juss., *Persicaria hydropiper* (L.) Spach. and *Vitex negundo* Linn over an exposure time ranging from 1 to 10 hour. In a similar study on repellency involving indigenous plants by Rehman et al. (2009), they reported on a 59% and 57% repellency from ethanol and petroleum extracts of these plants, respectively, against *Bactrocera zonata* (Saunder). In another study on repellency, Hidayat et al. (2013) reported that vegetable oils (safflower, cottonseed, linseed and neem oil) had lower RI_{50} values (the concentration that reduced fly landings by 50%) against gravid *Bactrocera tryoni* (Froggatt). Hence, vegetable oils had the repellent effect that kept female *B. tryoni* away from treated fruit Repellent effects of plant-derived extracts on other insect species have yielded similar results (Guo et al. 2016; Wu et al. 2015; Zapata & Smagghe 2010). Aryani and Auamcharoen (2016) reported on a strong repellency of the three Thai plants in the Zingiberaceae family against the maize grain weevil, *Sitophilus zeamais* (Motschlusky); these plants as potential repellent as plant-derived repellents are safe and less toxic (Talukder 2006).

Insects have a less complicated olfactory mechanism, whereby the periphery often comprises far smaller cells. The mammalian nose, on the other hand, contains several million olfactory sensory neurons (OSNs). Only thousands to tens of thousands of olfactory receptor cells are found on the antennas and maxillary palps of insects. Despite the lower number of OSNs, insects' sensitivity to particular odorants is by no means inferior to the mammalian olfactory system (Sachse & Krieger 2011). The olfactory senses of insects are activated by low concentrations of a wide range of volatile compounds at ambient temperatures (Richards & Davies 1977).

Most plants contain compounds, the primary functions of defending them against phytophagous (plant-eating) insects (Mithofer & Boland 2012; Schiestl 2010). Insects can detect odours when the volatile compound binds to odorant receptor (OR), often on the antennae and maxillary palps of the insect (Bohbot & Dickens 2010; Ditzen et al. 2008). Insects' ability to detect volatile compounds in the air creates an opening for more investigation into plants or plant products that negatively act on insects by repelling them away thus protecting crops. The findings from this study suggest that CNSL could be a potential candidate and hence to be part of the management strategy against *B. dorsalis*.

What stands out quite clearly in this study is that CNSL has the potential of being a viable pest control tool. It could be incorporated into the broad, integrated pest management strategy. Having CNSL as a stand-alone strategy is not a very strong option because of the fact that plant-derived insecticides degrade rapidly under field conditions due to UV light, temperature and moisture (Turek & Stintzing 2013), which may cause the lack of residual action for most botanicals (Isman 2006). However, despite this weakness, CNSL could be part of the pest management by the strategic distribution of impregnated fabric in the papaya plantation or schedule sprays with the liquid, which could be supported with a well carried out field sanitation. A well-timed spray with synthetic insecticide at the beginning of the season could help to eliminate any pest population from the previous season. The success of this approach will help in addressing the problem of insecticide resistance that has been observed in synthetic insecticides.

In most countries, especially the developing ones, farmers rely heavily on synthetic insecticides to control fruit flies populations. This is most concerning from the ecological point of view and the issue of insecticide residues in exported and locally consumed fruits such as papaya. Insecticides are meant to be toxic to specific groups of organisms; equally, they can also have profound adverse effects on the environment and other living organisms as well as on various forms of media, such as air, soil or water (Aktar et al. 2009; Bhat et al. 2019). Furthermore, some insecticides resist degradation and remain in the environment for years (Yadav et al. 2015). They can also be stored in human subcutaneous tissues, resulting in chronic poisoning (Eddleston 2019).

The hexane and dichloromethane extracts of CNS are potent and useful for *B. dorsalis* pest management as insect repellents. More studies are needed to identify the phytochemical compounds that are effective against *Bactrocera* species and other pests.

CONCLUSION

In view of the above results obtained in this study, it could be concluded that dichloromethane and hexane extracts of CNS have repellency effects on *B. dorsalis*. This indicates the potential use of CNSL in the field management of this destructive pest. Timely intervention with the extracts could prevent the female from laying eggs on the fruit and minimise the environmental and other health hazards. The results suggest that papaya treatment with the extracts may have limited activity as a stand-alone management tool for *B. dorsalis*. Hence, an integration between the use of the extracts and other management practices is required. Pest control using the botanicals extracts that influence the behaviour of the pest by repellence can be complementary to insecticide use because the extracts would not interfere with the insecticide mode of action. Furthermore, the botanical extracts can disrupt host insect interactions by repelling insects from a host, deterring feeding or egg-laying.

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