SLJoAS Sri Lankan Journal of Applied Sciences

Research Article

The Synergistic Effect of the Extracts of *Lantana Camara* L. and *Tithonia Diversifolia* (Hemsl) Against Grain Storage Pest of *Tribolium Castaneum* (Herbst)

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(Received 10th July 2023; Accepted 25th August 2023)

Abstract:

Insects cause extensive damage to stored grains and their products. Among the stored grain pests, *Tribolium castaneum* (Herbst.) is considered as one of the destructive pests. Since botanical insecticides are less hazardous to humans and the environment, they are frequently recommended for pest management during storage. This study was aimed at evaluating the synergistic effect of ethyl acetate extracts of *Lantana camara* L., and *Tithonia diversifolia* against *T. castaneum* (Herbst). The leaves were washed, air dried (27 ± 2 °C), and powdered before extracting them with ethyl acetate as a solvent. The activity of individual and combination of extracts were studied through different concentrations (0.2, 0.4, 0.6 µg/µL) against the adult beetles of *T. castaneum* to assess the contact toxicity and repellent activity. *L. camara* showed higher mortality and repellent activity over *T. diversifolia* and the positive control. However, the combination of both plant extracts (CE) significantly enhanced ($P < 0.05$) the mortality percentage and repellent activity over the individual crude extracts at tested concentrations. Therefore, it can be concluded that CE showed synergistic effect on toxicity and repellent activity against *T. castaneum*.

Key words: Mortality, Repellent activity, Synergism

1. Introduction

Cereal grains account for the bulk of commodities kept in storage and are an essential component of the world's food supply. However, there is a constant requirement for protecting grains during long-term storage from degradation, particularly loss of quality and weight caused by insect and beetle attacks. When they attack continuously, it becomes less marketable, resulting in economic loss and degradation of quality, which will affect consumers. *Tribolium castaneum* (Herbst) is one of the most common and harmful pests of stored foodstuffs, feeding on a variety of stored-grain and grain products [1]. Synthetic compounds used as pesticides, such as Dichlorodiphenyltrichloroethane (DDT), Malathion, Dichlorvos, and others, kill the storage pests by disrupting their nervous systems. However, there is growing concern about the negative effects caused by excessive use of these substances. A buildup of chemical residues on grains and lethal impacts on non-target creatures have resulted from the over use of these synthetic pesticides [2]. Based on these circumstances, an alternate strategy for decreasing stored-product losses by insects should be identified.

The use of plant-based insecticides as a replacement to synthetic insecticides to protect stored products is promising, owing to the ability to control environmental conditions inside storage units, maximizing insecticidal effect while being less toxic to humans and the environment [3]. Applying plant-based substances with insecticidal qualities are one of the most significant locally accessible, biodegradable, and low-cost pest management techniques for stored grains [1]. Among the plants, several plant species have been reported showing repellent and toxic effects to most of the grain storage pests including *T. castaneum* [4]. *Lantana camara* and *Tithonia diversifolia* have been identified as potential plant species that can be used as bioinsecticides against most of grain storage pests [5,6]. These are underutilized plants in Sri Lanka which are heavily used in conventional farming to eliminate insects. A study indicated that the leaf methanol extract of *L. camara* was toxic to most of grain storage pests including *T. castaneum*, showing *L. camara* as a potential source of biopesticide [7]. Green et al. have shown that the methanol extract of *T. diversifolia* showed contact toxicity to *Callosobruchus maculatusin.* a beetle attacking to cowpea, a dose dependent manner [8].

Previous studies have shown, polyherbal extracts prepared using different plant extracts produce synergistic effect that may increase the insecticidal and repellent activity than its individual plant extracts [9,13]. The synergistic usage of essential oils (cassia oil, lemongrass oil, eucalyptus oil, and rosemary oil) obtained from *Cinnamomum cassia*,

Cymbopogon citratus, *Eucalyptus citriodora* and *Rosmarinus officinalis* have been shown to repel *Aedes aegypti* more effectively than the individual essential oils [10, 11]. According to a study, when five plant extracts were combined (*C. citratus*, *L. camara*, *Calotropis gigantean*, *Ocimum tenuiflorum*, and *Azadirachta indica*), the effectiveness of the blended solution has increased while decreasing the evaporation of compounds which possess the repellent activity [12]. Synergism is the combination of toxicity between two substances combined and increased toxicities than the toxicities of individual substances [13]. Combination of insecticidal compounds show synergistic activity which can block insecticide detoxification, promote insecticide dispersion inside the cuticle, or act as a binding site for the target insect's receptor protein and thereby enhancing the effectiveness. Therefore, the present study was undertaken to analyze the synergistic effect of ethyl acetate extracts of *L. camara* L., and *T. diversifolia* against *T. castaneum* (Herbst) with the aim of developing a plant base insecticidal fumigant.

2. Materials and methods

2.1 Extracts of plant materials

The study was conducted in the year 2022 at the advanced research laboratory of Uva Wellassa University of Sri Lanka. The leaves of *L. camara* L. and *T. diversifolia* were collected from Badulla, Sri Lanka and authenticated by the National Herbarium of Royal Botanic Garden, Peradeniya, Sri Lanka. The leaves were washed and air dried at room temperature (27 ± 2 °C). Dried leaves were powdered and the extracts were produced by combining 100 g of each powdered leaf sample with ethyl acetate using the Soxhlet apparatus. The extracts were concentrated at 40 ºC in a rotating evaporator under reduced pressure. The extract was kept in pre-weighed vials in a freezer at -20 °C until it was employed in the bioassays. With ethyl acetate as a solvent, a range of concentrations of each extract (0.2- 0.6 µg/µL with 0.2 increments) were made. Combination of extracts (CE) were prepared by combining each extract (1:1 w/w) to have the final concentrations ranging from 0.2 to 0.6 µg/µL.

2.2 Rearing insects

Adult *T. castaneum* insects were collected and placed in a ventilated semi-controlled insect cage (average temperature of 27 ± 2ºC; ~70 % relative humidity (RH); photoperiod of L16:D8), where they were fed with wheat flour. Adult beetles were removed after 24 hours and left to incubate. In a vented container containing wheat flour, insect cultivation was maintained. After four to five days, the larvae were moved to separate Petri plates and fed wheat flour to develop the larva to adult beetles (14 days), and these laboratory-reared adult beetles were employed in the bioassay.

2.3 Contact toxicity

The experiment employed adult beetles that were 14 days old for each treatment and was set up in a fully randomized design (CRD) to assess five treatments including negative and positive controls with three replications. Adapting the topical application technique, the plant extracts were tested for contact toxicity [14]. Twenty mature beetles were placed in a Petri dish with a Whatman No. 1 filter paper disc for each test concentration. Each plate was treated with 20 µL of each plant extract separately using a micropipette. The positive and negative controls used were ethyl acetate and the insecticide Actellic (20%). After treatment, wheat flour was provided to the insects as food, and they were then given 10 minutes to dry at 27 ± 2 ºC. Beetles were tested for mortality after 48 hours; they were considered dead if they did not move after being gently probed for 15 seconds with a dissecting needle. Based on beetle mortality following treatment, the contact toxicity of solvent extracts was determined. LD_{50}, Chi-squared values, and beetle mortality information were derived using probit analysis using the Mini tab 2017 program. Analysis of variance (ANOVA) was used to compare each plant extract content, insect stage, and time observed from an assessment of insecticidal action. Using Abbott's technique, data were adjusted for mortality in the controls [15].

2.4 Repellent activity

The repellent assessment adopted a randomized controlled study design (RCD). The study used the area preference method [5]. Whatman No. 1 filter paper circles of 10 cm in diameter were cut in half to produce the two testing areas. A pipette was used to evenly apply one milliliter of each extract treatment, including the CE, at specified concentrations ranging from 0.2 to 0.6 µg/µL on a half filter paper disc. The treated test area of the studies was made by this half filter paper circle. To act as a negative control region, the opposite half circle was merely treated with solvent. The discs were then air dried to evaporate the solvents as much as possible. A conventional pesticide, Actellic, was administered to the treated area at the same dose range for positive control. The entire filter paper was then constructed by taping the treated half to the untreated halves. The treated and untreated half-circles were thus put adjacently on the Petri plates, and twenty beetles were carefully placed and covered at the center of each filter paper disc in the Petri dish. Each treatment was replicated four times. The number of beetles in the treated (Nt) and control (Nc) areas of preference was counted and recorded after every one hour for five hours. Insect repellent activity of the plant extracts was calculated in terms of percent repellent activity (PR) using the formula below.

$$PR = [(N_c-N_t)/N_c+N_t)] \times 100$$

where Nc is the number of insects recorded in the control half and Nt is the number of insects recorded in the treated half.

2.5 Data Analysis

At 48 hours after treatment, data on test beetle mortality were collected. Probit analysis yielded data on beetle mortality, Chi-squared values, and LD_{50} values, as well as the related 95% confidence intervals (95% CI). To calculate LD_{50} values, the relationship between the extract concentration used and percentage mortality was obtained using probit analysis. When their respective 95% confidence intervals (CI) did not overlap, LD values were considered to be substantially different. Data on mortality and repellent activity were evaluated in relation to dosage and response. From each of the several groups, the number of beetles on the two experimental sites (Nc and Nt) was gathered for the extract. Descriptive statistics were used to examine the outcomes of PR. Minitab version 17 software was used as the statistical program for all of these statistical studies. One-way ANOVA was used to analyze the data, and then Tukey's post hoc test for separation and pairwise mean comparisons were performed. and the 95% confidence interval was used to identify the significant differences.

3. Results and Discussion

3.1 Contact toxicity assay

All plant extracts had positive effects on the contact toxicity against adult beetles of *T. castaneum* in this assay (Table- 1). The ethyl acetate extracts of *L.camara* and CE gave very promising contact toxicity against *T. castaneum* over the treatments with *T. diversifolia*, NC and PC. The percent mortality increased with increasing concentrations, indicating that the effect of the all-plant extracts was dose-dependent. Out of the two plants tested, significantly the highest mortality percentage ($P < 0.05$) was recorded by *L. camara* in comparison with *T. diversifolia* and the positive control whereas the lowest was recorded by *T. diversifolia.* However, it was observed that the combined extract (1:1 w/w) (CE) significantly enhanced ($P < 0.05$) the mortality percentage over the other treatments under tested concentrations. The LD_{50} values were determined, where the CE had significantly the lowest value at all concentrations, being more toxic than the other individual leaf extracts (Table- 2). Although the crude extract of *L. camara* caused the second highest mortality at all concentrations, it was significantly different ($P < 0.05$) from the treatment with combined extracts. The treatment CE showed 22 % higher mortality percentage over the treatment *L. camara*. Based on probit analysis, *T. diversifolia* extract was the least toxic to *T. castaneum*. Any mortality was not recorded until 48 hours of exposure to ethyl acetate treatment (negative control), confirming that the solvent had no toxic effect on *T. castaneum*.

Table 1: The mean mortality (%) of individual and combined extracts on *T. castaneum*

Treatment	Concentration µg/µL	Mean Mortality % at 48 h
L. camara	0.2	48.9±2.3[a]
	0.4	57.8±1.5[a]
	0.6	76.7±3.7[a]
T. diversifolia	0.2	25.3±1.2[b]
	0.4	36.6±1.2[b]
	0.6	56.1±1.7[b]
CE	0.2	59.1±2.8[a]
	0.4	71.3±1.3[a]
	0.6	93.4±2.1[a]
PC	0.2	28.8±1.4[b]
	0.4	45.7±2.7[b]
	0.6	59.0±4.1[b]
NC		0.0[c]

All values are means of triplicate determination ± SD
Different letters in the same column showed statistical difference according to Tukey's test at $P < 0.05$

Table 2: Statistical comparison of LD_{50} values of plants- extracts against *T. castaneum* tested by topical application.

Plant	LD_{50}	95% CI	Slope (±SE)	Chi-squared (DF = 2)	P-value
CE	110.3	86.3-122.4	1.04 (0.10)	8.9	0.00
L.camara	137.7	97.7-164.1	1.12(0.12)	9.1	0.03
T. diversifolia	332.1	309.6-368.7	1.40(0.16)	18.8	0.60
PC	280.7	250.1-312.6	1.22(0.15)	14.4	0.08

Scientists are more interested in evaluating the bioactivity of plant extracts against pest insects that attack stored grains [16]. The current investigation found that the leaf ethyl acetate extracts of *T. diversifolia and L. camara* showed toxicity against *T. castaneum* (Tables 1 and 2). Out of the two tested plants, the ethyl acetate extracts of *L. camara* displayed the highest mortality percentage whereas *T. diversifolia* displayed the lowest at all tested concentrations. Studies have shown *L. camara* contains phytol, pyrroline, 1-dodecanol, paromomycin, 1-hexacosanol, and amphetamine as the major chemical constituents [17]. Further it has been shown that these chemical constituents can rapidly penetrate into insects [16] and generated toxic action. Pyrroline is an active biochemical molecule that possesses insecticidal and fungicidal properties when it enters insects, causing biochemical malfunction and mortality. Insecticidal properties have been reported for the substances such as dodecanol, pyrrolizin, paromomycin, 1-docosene, and nonadecene [18]. The fumigant toxicity of crude extract *L. camara* against rice weevils *Sitophilus oryzae* has been

documented, and the mortality of *S. oryzae* has risen with increasing exposure duration and extract concentration [19]. Our study showed a similar result confirming the dose dependent manner of the mortality with the time and the extract concentration. In contradictorily, it has been reported the less effectiveness of the methanolic extracts of *L. camara* on *T. casatneum* [7]. Ethyl acetate extracts of the leaves, stems, and roots of *T. diversifolia* have shown significant inhibition of wheat coleoptile growth [20]. Oyedokun and co-workers reported that aqueous and ethanolic extracts of *T. diversifolia* showed insecticidal activity on *Macrotermes bellicosus* (Termitidae) [21]. Furthermore, a dichloromethane extract of *T. diversifolia* leaves at a concentration of 0.001 µg/µL exhibited the highest insecticidal efficacy, killing 70 % of *Atta cephalotes*, worker ants after five days of treatment [22]. In contradictory, ethyl acetate extract of *T. diversifolia* showed the least mortality (56%) on *T. casatneum* even at 0.6 µg/µL concentration in our study.

The combination of two extracts, on the other hand, increased motility with the lowest LD_{50}, showing that *T. castaneum* is more responsive to the synergistic action of the two plant extracts. The findings of the current study are consistent with the previous studies showing the synergistic effects of different plant extracts on insect mortality. Synergistic contact toxicity effect has been reported when the clove oil was mixed with the sesame oil at the ratio of 8:2 against *Callosobruchus maculatus.* After 48 h of exposure, a higher insect mortality has shown by that mixture over their individual treatments [23]. A binary mixtures of *Piper retrofractum* and *Acorus calamus* has shown synergistic effects, suggesting that these mixtures could serve as an acute toxicant against *Spodoptera litura*. In particular, the mixture of *P. retrofractum* + *A. calamus* (3:1) at this combination has exhibited the highest antifeedant activity at 82.43%, showing synergistic contact toxicity effects [24]. Another study revealed that a combination extract (3:7) of *Azadirachta indica* and *Moringa oleifera* was more poisonous to *Nilaparvata lugens* than either compound alone [25].

3.2 Repellent activity

The insect repellent activity of two plant extracts and its 1:1 (w/w) combined extract with varying concentrations at different time intervals is shown in Figure 1 (a,b,c). The results revealed that the concentration of the extracts of all plants used had a significant influence on the degree of repellent activity, as it resulted in a consistent pattern of repellent activity from the lowest to the highest dosages. It was noticed that there was progressively increased PR against *T. castaneum* with increasing concentration and exposure time in all treatments. Out of two plant extracts, significantly the highest ($P < 0.05$) PR was observed in *L.camara* over the *T. diversifolia* and PC at all the time intervals. The lowest PR was observed by *T. diversifolia* at all time intervals in all the extract concentrations. However, it was clearly observed that by combining the two plant extracts (CE), the PR was enhanced at all time intervals in all concentrations. At higher concentrations (4 and 6 µg/µL), CE showed 100 % repellent activity against *T. castaneum* at all the time intervals (Fig. 1b and c). A significant difference was observed in PR between the CE and the extract from *L. camara* against *T. castaneum* at lower exposure time periods. However, PR occurred by *L. camara* reached to 100% with the increment of the exposure time. Although, the rate of the increment of PR in both CE and *L. camara* against *T. castaneum* increased with the time until reaching 100 % repellent activity, it was gradually decreased with the time in both PC and *T. diversifolia*. The treatment *T. diversifolia* showed considerable decrement in PR after 4 hours of exposure time at all concentrations. The solvent had no effect on repellent activity of *T. castaneum*.

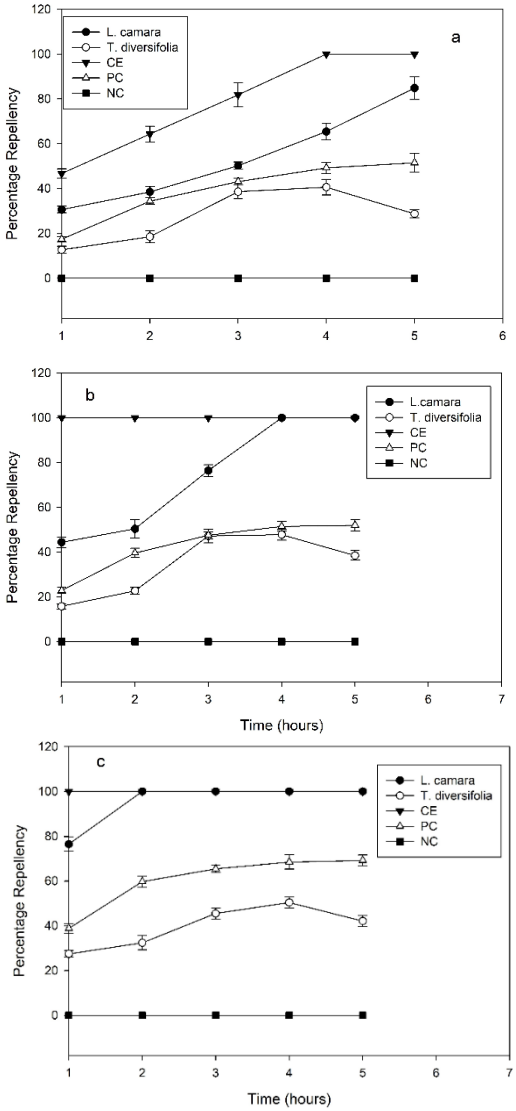

Figure 1- The effect of plant extracts on repellent activity percentage against *T. castaneum* with time under different extract concentrations. a) at 2 µg/µL b) 4 µg/µL c) 6 µg/µL

It has been reported that the leaf powder of *L. camara* showed significant repellent activity against maize weevil of *Sitophilus zeamais*. The dosage of powder used and the exposure time have a significant impact on the level of

repellent activity. As the powder dose rose from 2 % (w/w) to 10 % (w/w) and the exposure duration extended from 6 h to 24 h, the percentage of *S. zeamais* repellent activity against *L. camara* leaf powder increased from 30 % to 90 % [7]. It has been reported that, exposed to *S. zeamais* weevils for the second and third hours, a 100 % dosage of *T. diversifolia* extract was said to have a 100 % repellent effect [6]. The same study discovered that the percentage repellent activity of *T. diversifolia* ethyl acetate extract dropped when dose concentration was reduced. The current study is consistent with the previously reported data confirming the high insect repellent activity of ethyl acetate extract of *L. camara* (100% repellent activity after 2 hrs of exposure for 6 µg/µL concentration). Further, the current study also reported that the concentration of both plant extracts and the exposure time of the extracts had a significant influence on the degree of repellent activity.

The current study showed a significant synergistic repellent activity by CE of both *L. camara* and *T. diversifolia* over their individual extracts at 6 µg/µL concentration. Repellent properties of the extracts are typically attributed to its chemical constituents present. A synergistic phenomenon has been noticed that might emerge between its chemical constituents and leading to a greater bioactivity than the individual components [26]. When neem oil and lantana oil extracts were evaluated for their effectiveness in repelling mosquitoes, it was shown that the combination had higher repellent activity than either extract alone, which might have extremely low repellent activity [27]. Another study indicated that a 1:1:2 essential oil blend of *Curcuma longa* rhizomes, *Pogostemon heyneanus* leaves, and *Zanthoxylum limonella* fruits provided higher protection against *Ae. albopictus* mosquitos than their respective plant extracts [28]. Furthermore, a 1:4 mixture of extracts of *Vetiveria zizanioides* and *Andrographis paniculata* demonstrated significantly stronger escape patterns in the laboratory strain compared to individual extracts, while a 1:1 mixture of *Andrographis paniculata* and *Cananga odorata* demonstrated strong irritant activity against the field strain of *Ae. aegypti* [29]. This confirms the synergistic effect of plant extracts on insect repellent activity and mortality over the individual extracts.

4. Conclusion

L. camara L. and *T. diversifolia* have insecticidal activity against *T. castaneum*. The highest significant contact toxicity and repellent activity were recorded by the 1:1 (w/w) combination of ethyl acetate extracts of *L. camara* and *T. diversifolia* Therefore, this study revealed that the combination of both plant extracts has a synergistic effect against *T. castaneum*. However, further investigations should be carried out to evaluate whether new active compounds are produced in the combined extract. Further, isolation of active compounds followed by bio assays are essential to identify the active compounds.

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