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EVALUATION OF THE INSECTICIDAL PROPERTY OF *Lansium domesticum* Correa FRUIT PEEL AND SEED EXTRACTS AGAINST ARMY WORM (*Spodoptera frugiperda* J.E. Smith) AND ASSESSMENT OF THE CYTOGENOTOXIC EFFECTS ON *Allium cepa* L.

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ABSTRACT – The present study evaluated the insecticidal property of *Lansium domesticum* fruit peel and seed extracts against army worm (a known pest of onion) using susceptibility test at 24 and 48 hours of incubation. In addition, the LC_{50} values were determined using Probit's analysis. Cytogenotoxic effects of *Lansium domesticum* seed and fruit peel aqueous extracts on *Allium cepa* root tip cells were assessed by determining the mitotic cell index and chromosomal aberrations. Lastly, phytochemical analysis of *L. domesticum* extracts was performed. Susceptibility test showed the insecticidal property of *L. domesticum* fruit peel ($2.5x10^4$ ppm) and seed extracts ($2.5x10^4$ and $1.75x10^4$ ppm) against army worm. After 48 hrs of incubation, army worm treated with *L. domesticum* seed and fruit peel extracts ($2.5x10^4$ ppm) registered mortality of 80.95% and 66.67%, respectively. The computed LC_{50} value of *L. domesticum* seed extract was at $1.5x10^4$ ppm and $3.2x10^4$ ppm for *L. domesticum* fruit peel extract. Results also revealed the cytotoxic and genotoxic effects of *L. domesticum* on *A. cepa* root tips cells. The mitotic cell index of onion root tip cells of 76.40% (control) was reduced to 62.66% and 60.40% when treated with $2.5x10^4$ ppm of *L. domesticum* fruit peel and seed extracts, respectively. In terms of genotoxicity, chromosomal aberrations such as nuclear lesions, vagrant, laggard, polyploidy, and binucleated cells were observed in onion root tip cells treated with *L. domesticum* extracts with the percentage incidence of chromosomal abnormalities of 12.74% (cells treated with seed extracts) and 7.53% (cells treated with fruit peel extracts). Phytochemical analysis showed that *L. domesticum* fruit peel and seed extracts contain essential oils, steroids, phenols, tannins, and flavonoids, while alkaloid was only detected in seed extracts. Thus, *L. domesticum* seed and fruit peel extracts are potential botanical insecticides against army worm with negligible cytogenotoxic effects on *Allium cepa*.

Keywords: cytotoxicity, genotoxicity, insecticide

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INTRODUCTION

Lansium domesticum Correa (Meliaceae) is commonly known as lanzones and a native of Southeast Asian countries (Ragasa et al., 2006). In the Philippines, it is mostly grown in Southern Luzon and Mindanao. *L. domesticum* has been known for the distinct characteristics of its fruit which is a sweet flesh, bitter-tasting seeds, and thin fruit peel with latex secretion. Its fruit contains vitamins, protein, electrolytes, minerals, and carbohydrates. In addition, its fruit peel and seeds have long been utilized as arrow poison and insect fumigant or botanical insecticide, which is cost- effective, environmental-friendly, and non-toxic to mammals. Accordingly, the insecticidal property of *L. domesticum* can be attributed to the presence of triterpenoids (lansic acid and lansiosides), tannins, and alkaloids (Marfori et al., 2015).

Onion *(Allium cepa* L.) is one of the important crops worldwide and in the Philippines, it is mostly cultivated in the Ilocos region, Tarlac, Pangasinan, and Nueva Ecija. However, one of the problems in cultivating onions is their vulnerability to certain crop diseases and insect pests such as army worms. In 2016, army worms infested thousands of hectares of onion fields in Tarlac, Nueva Ecija, and Pangasinan where most of the onions are produced (Alberto et al., 2019). The larvae of army worm bore into the leaves while leaving the exterior part of the plant intact, and its resistance to numerous commercial pesticides caused its outbreak (Ueno, 2015).

Aside from its importance in the field of agriculture, onion is also known as a model plant for cytogenetoxic potential of various pollutants due to the sensitivity of root growth dynamics to pollutants, stable karyotype and chromosome number, and diverse morphological characters (Firbas & Amon, 2014; Fiskesjo, 1997). Studies had revealed the genotoxicity of pesticides which drastically induce chromosomal aberration and decrease cell division of plants. They are found to be genotoxic, as they decrease the mitotic index and increase chromosomal aberration (Gadano et al., 2002; Kura et al., 2006). Also, insects can easily develop resistance to synthetic pesticides whereas, the resistance to botanical pesticides can be hindered by its behavioral and physiological pesticidal actions (Rattan, 2010).

With the destructive effect of the army worm on crops and massive threat on our food security, there is a need to develop biological control to prevent losses in crop production and synthetic insecticide should be our last resort. Various studies had already been conducted in search for biocontrol against armyworm. In this study, the insecticidal potential of *L. domesticum* seed and fruit peel aqueous extract against army worm was investigated. In addition, the cytogenetic effect of *L. domesticum* fruit peel and seeds was assessed using *A. cepa* both as the host plant for army worm and the model plant for the chromosome aberration assay.

MATERIALS AND METHODS

Fruit peel and seed collection and extraction

L. domesticum fruit peel and seeds were obtained from the local growers of Los Baños, Laguna, Philippines. Fruit peels were rinsed, cut into small pieces, and air-dried at room temperature for seven days. Then the fruit peels were pulverized using a blender, extracted in 300 ml of distilled water, maintained in water bath at 80-90°C for 2 hours, filtered and was kept refrigerated until use.

Susceptibility test for army worm in L. domesticum extracts

Susceptibility test was carried out to determine the potential insecticidal/larvicidal activity of the *L. domesticum* fruit peel and seed aqueous extracts against the 3rd instar larvae of army worm. During the

3rd instar stage of larvae of army worm, the larvae start to infest the crops by making holes and eating the leaves of the infested crops. The 3rd instar larvae of army worm were obtained from National Crop Protection Center, University of the Philippines, Los Baños, Laguna.

Each 3rd instar larvae of army worm was placed in a separate container and was fed with young corn leaves soaked in different treatments [(Peel extracts and seed extracts of *L. domesticum* ($2.5x10^4$ ppm and $1.75 x10^4$ ppm)], distilled water as negative control and commercial pesticide for positive control). The commercial pesticide used contains acylurea which acts as growth regulator by inhibiting the chitin synthesis and preventing the molting of the larvae. Ten larvae were used for each treatment and were replicated three times. Mortality rates were observed and computed at 24 and 48 hours of incubation.

Allium cepa Assay

Locally produced onion was purchased from the onion storage of San Jose, Nueva Ecija, Philippines. *A. cepa* rooting was initiated using a root-dip technique in 50 ml distilled water for two days. Then the rooted bulbs were transferred into a beaker filled with different treatments for 24 hours.

Cytological studies

Onion root tips were hydrolyzed in 1 M of HCl for 10 minutes and washed three times with distilled water. The specimen was pressed on each slide and stained with acetocarmine for 15 minutes (Yekeen et al., 2017). Then the slide was observed under the light microscope (Cole Parmer B2-Series), 40x magnification. The number of cells in different mitotic stages were counted and the total mitotic index and mitotic inhibition were calculated. All cells with chromosomal aberrations such as chromatid damage, chromosome damage, and chromatid type damage were counted, and each abnormality was photographed. The aberration incidence was computed by dividing the number of total chromosome aberrations into total number of mitotic indices multiplied by 100.

Phytochemical Screening of L. domesticum seed and fruit peel extract

Phytochemical screening was carried out for each plant extract to detect the secondary metabolites present. Samples were sent to St. Mary's University for evaluation. The following protocol was adapted for the phytochemical screening.

Each plant extract was spotted on mark, labeled, and subjected to TLC (Thin Layer Chromatography). The 7x4 cm plate was developed in the acetate-methanol (7:3) solution. The spots for the certain metabolite were visualized on the TLC plates and exposed under UV light and hot plate to check the separation of different compounds. For typical visualization of secondary metabolites, a vanillin-sulfuric acid reagent was utilized. This solution can determine the presence of phenols, sterols, triterpenes, and essential oils. Methanolic potassium hydroxide was used to test anthraquinones, and anthrones, while phenolic compounds and tannins were detected using potassium ferrycyanide-ferric chloride reagent. Dragendorff's reagent was used to spot alkaloids, and antimony (III) chloride was used to detect the presence of flavonoids (Kolak et al., 2006).

Statistical Analysis

The study was laid out using Completely Randomized Design (CRD). Data were analyzed using Analysis of Variance (ANOVA) and Comparison among Means by *Tukey's HSD* (honestly significant difference) *test*. All tests of significance were done at 5% probability levels.

RESULTS and DISCUSSION

The study was divided into two parts, the first part focused on the insecticidal activity of the fruit peel and seed aqueous extracts against army worm and the second part determined the cytogenetoxic effect of the extracts in onion root tip.

Insecticidal Activity of L. domesticum seed and fruit peel aqueous extract

The mortality rate of the 3rd instar larvae of army worm is shown in Table 1. Results showed 100% mortality of army worm treated with commercial pesticide, followed by seed extracts ($2.5x10^4$ ppm) with 47.62%, and army worm treated with the fruit peel extract ($2.5x10^4$ ppm) with 33.33%. Meanwhile, at 48 hrs of incubation, high rates of mortality were observed in army worm treated with seed extracts ($2.5x10^4$ ppm and $1.75\ x10^4$ ppm) of 80.95% and 66.67%, respectively. In addition, the LC_{50} value of seed extract against army worm were computed at $1.50\ x10^4$ ppm and $3.2\ x10^4$ ppm for the fruit peel extract. The mortality rate of army worm treated with commercial pesticide is statistically higher compared to all the treatments, while a significant increase in mortality rate of army worm was observed when treated with *L. domesticum* peel and seed extracts at 24 and 48 hrs of incubation.

Table 1. Mortality rate of 3rd instar larvae of army worm at 24 and 48 hrs of incubation.

TREATMENTS	24hrs	48hrs
Distilled water	0^{d}	0^{d}
Commercial Insecticide	100^{a}	100^{a}
Peel extract ($2.5x10^4$ ppm)	33.33^{bc}	57.14^{cd}
Peel extract ($1.75\ x10^4$ ppm)	4.76^{cd}	47.62^{d}
Seed extract ($2.5x10^4$ ppm)	47.62^{b}	80.95^{b}
Seed extract ($1.75\ x10^4$ ppm)	23.81^{bc}	66.67^{c}
LC50 value		
Seed extract		$1.49x10^4$ppm
Fruit peel extract		$3.18x10^4$ ppm

*Means in column with different letters are significantly different at 0.05 level of significance.

The mortality of larvae due to pesticides is caused by the inhibitory action on the adenosine triphosphatases, acetylcholinesterase, butyrylcholinesterase, opamins, and gamma aminobutyric acid receptors which in turn affect the pest's resistance to pesticides by inhibiting the secretion of detoxifying enzymes (Ebadollahi et al., 2013; Ozbek et al., 2017; Gunderson et al., 2018; Jankowska et al. 2018). The active chemical ingredient acylurea also contributed to the mortality of the army worm (Osman et al., 2016; Plata-Rueda et al., 2017).

The insecticidal activity of the *L. domesticum* seed and fruit peel extracts can be attributed to the presence of several phytochemicals which are presented in Table 4. As mentioned by Kostyukovsky et al. (2002) and Kumar et al. (2015), the effect of the combination and interaction of secondary metabolites are a deterrent and toxic to insects and herbivores for longer periods than single compound. Smilanich et al. (2009) and Regnault- Roger & Philogene (2008) named these secondary metabolites as plant insecticidal toxins which can slow down insect growth rate and indirectly reduce feeding performance. Accordingly, alkaloids appear to disrupt insect hormone balances, preventing molting, and causing death. They can also

prolong the development of the third instar to emergence (Sun et al., 2012). In addition, alkaloids serve as protection against herbivores due to its bitter flavor which can cause protein disruption and can lead to central nervous system alteration (Acheuk & Doumandji-Mitiche, 2013). Moreover, essential oils have larvicidal, repellent, insecticidal, antifeedant, growth inhibitor, oviposition inhibitor, ovicide, and growth-reducing effects on a variety of insects (Shelton et al., 2002; Tripathi et al., 2003; Pereira et al., 2006; Sithisut et al., 2011; Regnault-Roger et al., 2012; Osman et al., 2016; Said-Al Ahl et al., 2017). Eugenol, isoeugenol, methyleugenol, methyl isoeugenol, coumarin, coniferyl aldehyde, diniconazole, ethyl cinnamate, and rosmarinic acid are among the compounds of essential oils that possess pesticidal properties (Shukla et al., 2012; Vidyasgar et al., 2012; Aref et al., 2015; AlJabr et al., 2017).

Cytotoxic effect of L. domesticum seed and fruit peel extracts

Mitotic index is used for the estimation of the frequency of cellular abnormalities and reduction of mitotic activities. Cytotoxic substances both synthetic (such as pesticide) or naturally occurring in nature can also be detected through this assay (Asita & Mokhobo, 2013).

The cytotoxic effect of *L. domesticum* seed and fruit peel extracts were evaluated using the *Allium cepa* root tip meristem. The mitotic indices and mitotic inhibition are presented in Table 2. Untreated *A. cepa* recorded the highest mitotic index of 76.40%, followed by *A. cepa* seed extract with the higher mitotic index of 62.6%, and *A. cepa* treated with fruit peel of 60.40% which are statistically higher compared to the commercial insecticide (41.73%). This led to a high mitotic inhibitory activity of 45.45% in *A. cepa* treated with commercial pesticide, followed by the *A. cepa* treated with *L. domesticum* seed extract of 21.05%, and the least of 18.08% in *A. cepa* treated with *L. domesticum* fruit peel. Statistical analysis showed that mitotic inhibition in *A. cepa* treated commercial pesticide is significantly higher compared to *A. cepa* treated with *L. domesticum* extracts.

Synthetic pesticides have been well documented to cause genotoxic effect inducing significant reduction in mitotic cell index of organisms (Flessel et al., 1993; Giri et al., 2002). Mitodepressive and cytotoxic effects of the pesticide and *L. domesticum* by the inhibition of DNA were implicated with the reduced mitotic index (Akinboro et al., 2011; Nefic et al., 2013). Similarly, pesticides interfere in the normal process of mitosis that may affect enzyme production or enzyme function, induction, repression, or feedback inhibition. This could be a possible reason for the decreasing mitotic index by reducing the number of the dividing cells (Kwong 2002; Lamsal et al., 2010). Furthermore, Azeez et al. (2016) and Hikal et al. (2017) revealed that the presence of some bioactive compounds in nature are cytotoxic which cause mitotic suppression. These metabolites also include saponin, flavonoids, and steroids among others that are found in the tested medicinal plants.

Table 2. Percent mitotic indices and mitotic cell inhibition of *A. cepa* root tip meristem cells treated with fruit peel and seed extracts of *L. domesticum.*

	Mitotic cell index	**Mitotic cell inhibition**
Distilled water (negative control)	76.40[a]	
Commercial insecticide	41.73[c]	62.66[b]
Fruit peel extract	60.40[b]	18.08[b]
Seed extract (2.5 x 10^4 ppm)	62.66[b]	21.05[b]

*Means in column with different letters are significantly different at 0.05 level of significance

Genotoxic effect of L. domesticum fruit and seed extract

Chromosomal aberrations as well as nuclear aberrations are considered as end result of genotoxic effects of various physical and chemical agents, and several studies showed the induction of chromosomal aberrations by pesticides (Masood & Malik, 2013). These indicate the mutagenicity of pesticides for non-target organisms and their effects on ecosystems are of concern worldwide (Asita & Mokhobo, 2013).

Chromosomal aberrations with physiological effect (vagrant and laggard) and clastogenic effect (chromosomal break, bridges) were found both in seed and peel extract. As presented in Table 3, *A. cepa* treated with seed extract recorded an aberration index of 12.74% which is comparable to the *A. cepa* treated with fruit peel extract with 7.53%. Chromosomal abnormalities observed include polyploidy, nuclear lesions, chromosomal fragments, vagrant, and binucleated cells. In addition, compared to the chromosomal aberrations recorded in commercial insecticide (57.95%), these values were significantly lower, which indicate that *A. cepa* treated with *L. domesticum* extracts are safe with minimal mutagenic effect to the onion plant. Accordingly, botanical pesticides are safer than synthetic pesticides. The latter is known to contribute to ozone depletion, releasing neurotoxins, carcinogens, teratogens, and mutagens to non-target organisms, as well as to the host plants, which affects plant growth and development (Regnault-Roger et al., 2012; Plata-Rueda et al., 2017).

Table 3. Chromosomal aberrations incidence of *A. cepa* root tip meristem cells treated with fruit peel and seed extracts of *L. domesticum*.

	Polyploid	**Nuclear lesions**	**Chromosomal Fragments/ Bridge**	**Vagrant/ Laggard**	**Binucleated cell**	**% Aberration indices**
Distilled water	1.33	0	0	0	1.33	0.48^{c}
Commercial Insecticide	66	24.67	0.67	2.67	24.5	57.95^{a}
Fruit peel extract	0	3.33	0	0.67	6.67	7.53^{b}
Seed extract ($2.5x10^4$ ppm)	22.67	6.67	0	0	5.33	12.74^{b}

*Means in column with different letters are significantly different at 0.05 level of significance.

Chemicals have depressive effects on the mitotic index and chromosomal aberrations like stickiness, laggards, chromatin bridges, C metaphase, fragmentation, and binucleate cells (Kumar and Tripathi 2003; Mallikarjuna et al., 2004; Sreedevi & Bindu, 2004). These can lead to a low accumulation of energy which result to the sticky nature of chromosome, causing delayed terminalization and failure of chromosome movement and the incidence of abnormalities (Fiskesjo, 1997; Azeez, 2016). Laggard and vagrant chromosomes arise mainly due to abnormal spindle formation from centromere adhesion causing abnormality of chromosome movement towards the equatorial, wherein the spindle fiber fail to carry the respective chromosomes to the polar regions (Grant, 1982; Kura et al., 2006; Pulatea & Tarar, 2014). Meanwhile, the presence of chromosome bridge is caused by breakage and fusion of chromosomes and chromatids, failure of free anaphase separation and unequal translocation or inversion of chromosome segments (El-Ghamery et al., 2005), during which chromosomes fail to separate because of chromosome stickiness (Yadav, 1986). Moreover, according to Nefic et al. (2013), nuclear abnormalities are morphological alterations in the interphase nuclei which include nuclear buds, cells without nucleus–ghost cells, fragmented nuclei, and apoptotic bodies which may lead to aneuploidy and then to cell death.

Similarly, alterations in cell shape and size are noted. The presence of fragmented nuclei and polynuclear cells can indicate a cell death process.

Phytochemical composition of L. domesticum seed and fruit extract

The aqueous extract of *L. domesticum* seed and fruit peel were subjected to phytochemical analyses to establish the presence or absence of essential oils, triterpenes, steroids, phenols, sugar, fatty acids, tannins, flavonoids, and alkaloids. Results of phytochemical analyses are presented in Table 4. Both *L. domesticum* seed and fruit peel aqueous contains essential oils, steroids, phenols, tannins, and flavonoids. Meanwhile, alkaloid was only detected in *L. domesticum* seed.

Based on several researches, secondary metabolites possess insecticidal properties and can be used as an excellent alternative to synthetic pesticides (Regnault-Roger & Philogene, 2008; Sithisut et al., 2011). Some steroids- based compounds can mimic actions of hormones which can be considered as safe insecticides (Fan et al., 2015). Meanwhile, flavonoids-based pesticides have been developed due to its ability to inhibit enzymatic activity and prevent the growth of larvae of different insect species (Kim et al., 2000). Also, flavonoids can interfere and inhibit in the process of molting, formation of ecdysone, and reproduction of several insects (Oberdorster et al., 2001). Tannins, on the other hand, are endogenous inhibitors of the growth of numerous species of pests by acting as anti-nutritional deterrent against insects and aphids due to oxidation mechanisms forming semiquinone radicals and quinones (Barbehenn et al., 2008; Isman & Grieneisen, 2014; D' Incao et al., 2013). Alkaloids such as furocoumarin, quinolone, pipernonaline, and piperidine are some of the plant-based alkaloids which showed larvicidal and antifeedant activities (Emam et al., 2009; Rattan, 2010; Acheuk & Doumandji-Mitiche, 2013; Wachira et al., 2014; Velu et al., 2015). Both flavonoids and isoflavonoids are known as phytotoxic compounds (Simmonds & Stevenson, 2001; Simmonds, 2003; Schuier et al., 2005; Gould & Lister, 2006). Plant essential oils are a mixture of terpenes that exhibit pesticidal activities which can be sublethal to lethal depending on the concentration and doses (Pavela & Benelli, 2016; Bakkali et al., 2008; Isman et al. 2011; Regnault-Roger et al. 2012; Campos et al., 2019; Ebadollahi et al., 2020). Previous studies have already documented the pesticidal effects of essential oils extracted from different plant families (Rajendran & Sriranjini, 2008; Ebadollahi & Jalali, 2015; Bahrami et al., 2016).

Table 4. Phytochemical composition of *L. domesticum* seed and fruit peel extract.

Phytochemical composition	***L. domesticum* aqueous extract**	
	Seed	**Fruit peel**
Essential oils	+	+
Triterpenes	-	-
Steroids	+	+
Phenols	+	+
Sugar	-	-
Fatty acids	-	-
Tannins	+	+
Flavonoids	+	+
Alkaloids	+	-

Note: (+) present; (-) absent.

In line with these findings, further studies must be done to be able to elucidate the compounds responsible for the pesticidal property and to test further the efficacy of the *L. domesticum* fruit peel and seed extracts.

CONCLUSIONS

Based on the results of the study, it can be concluded that *L. domesticum* aqueous seed and fruit peel extracts are potential botanical pesticides against army worm with computed LC_{50} value of $1.5x10^4$ ppm (*L. domesticum* seed extract) and $3.2x10^4$ ppm (*L. domesticum* fruit peel extract). Additionally, their cytotoxic and genotoxic effects on *A. cepa* root tips cells are negligible with reduction on the mitotic cell index which were significantly lower as compared to those treated with synthetic insecticide. The observed insecticidal activity of *L. domesticum* fruit peel and seed extracts can be attributed to the presence of essential oils, steroids, phenols, tannins, and flavonoids, and alkaloid in seed extracts.

STATEMENT OF AUTHORSHIP

The main author and the second author conceptualized, implemented, and wrote the research article. The third author supervised, reviewed, and provided critical feedback for the improvement of the paper.

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