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Insecticidal activity of wood vinegar mixed with *Salvia leriifolia* (Benth.) extract against *Lasioderma serricorne* (F.)

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Abstract. Insecticidal properties of wood vinegar on the activity of aqueous, methanol, and ethanol extract of *Salvia leriifolia* (Benth.) (Lamiaceae) were determined by measuring the mortality of cigarette beetle, *Lasioderma serricorne* (F.). Wood vinegar itself did not show insecticidal activity on *L. serricorne*. When the insect was treated with wood vinegar mixed with root, leaf, and stem extracts the mortality induced by methanol extracts was greatly increased by the wood vinegar in comparison with a single methanol extracts treatment. Wood vinegar showed antagonistic effect on aqueous and ethanol extracts. These results suggest that wood vinegar has a synergistic effect on the insecticidal activity of methanol extracts. Our study provides information on a potential role of wood vinegar in facilitation of activity of specific plant extracts.

Key words: *Salvia leriifolia*, plant extract, wood vinegar, *Lasioderma serricorne*.

Introduction

Methods used to control stored grain insect pests include physical, chemical, and biological treatments. Chemical treatment is a widely used method of control. Effective procedures include the use of fumigants or grain protecting. The chemical used should leave no hazardous residues and should not adversely affect the nutritional quality, flavor, or processing characteristics of the grains (Lee et al. 2002). Pest control in many storage systems depends on fumigation with either methyl bromide or phosphine. The use of methyl bromide is being restricted because of its potential to damage the ozone layer (Butler & Rodriguez 1996, MBTOC 1998). The use of phosphine (PH_3) is increasing due to the convenience of formulations, the relatively short–term hazard, and low retention of residues. However, PH_3 fumigation may become increasingly limited in use because resistance of stored grain insects to phosphine has now been discovered in more than 45 countries (Bell & Wilson 1995, Chaudhry 1995).

Under these circumstances, the need to search for a self, economic and viable alternative is growing. In these respect, we have been looking for alternative material in the plant kingdom with an emphasis on the extracts from plant grown in Iran. Scientists are now experimenting and working to protect insect infestation by indigenous plant materials. *Salvia leriifolia* (Benth.) is a perennial herbaceous plant that grows exclusively in south and tropical regions of Khorasan and Semnan provinces, Iran. This plant introduced in Florica Iranica in 1982 and has different vernacular names such as Nuruozak and Jobleh (Rechinger 1982).

Wood vinegar is a byproduct from charcoal production. It is a liquid generated from the gas and combustion of fresh wood burning in an airless condition namely, Iwate kiln. When the gas from the combustion is cooled, it condenses into liquid. Wood vinegar has been used in a variety of processes, such as industrial, livestock, household and agricultural products (Apai & Thongdeethae 2001). Raw wood vinegar has approximately 200 chemicals, such as acetic acid, formaldehyde, ethyl–valerate, phenol, methanol, tar, etc (Yoshimura & Hayakawa 1991).

In the aspect of plant protection, the wood vinegar has been used for antibacterial and antifungal properties (Seo et al. 2000, Radhakrishnan et al. 2002, Nakai et al. 2005), it has also been reported to have termiticidal and insecticidal activities (Yatagai et al. 2002, Kim et al. 2008, Pangnakorn et al. 2011). This report describes a laboratory study to assess the potential of wood vinegar with *S. leriifolia* extracts for use as commercial insecticides against *L. serricorne*.

Material and methods

Insect culture

Lasioderma serricorne was reared in glass container (1 litter) containing wheat flour and covered with a fine mesh cloth for ventilation. The culture was maintained in the dark in an incubator set at 27±2°C and 60±5% RH. Parent adults were obtained from laboratory stock cultures maintained at the Entomology Department, University of Urmia, Iran. Adult insects, 7–14 days old, were used for fumigant toxicity tests.

Plant material

Root, leaf and stem of *S. leriifolia* were collected from plants growing wild in Razavi Khorasan Province, Northeast of Iran region of Estaj (35° 55' N; 57° 37' E; 1700 m a.s.l), 250 km of Mashhad, Iran. Plant materials were collected at the full flowering stage during the month of May 2011. Plant taxonomists in the Department of Biology at Urmia University, confirmed the taxonomic identification of plant species. Voucher specimens were deposited at the Department of Plant Protection, Urmia University, Urmia, Iran. Plant materials were air dried for 14 days at room temperature (23–24 °C).

Extraction

Extractions of plant were investigated according to the method of Hosseinzadeh et al. (2007). For preparation of aqueous extract, the powdered root, leaf, and stem (100 g) boiled in 1000 ml boiling water for 15 min. Subsequently, the mixture was filtered and concentrated under reduced pressure at 35°C. As some constituents are sensitive to boiling water, a macerated extract was also prepared. For preparation of the solvents (methanol and ethanol: 96% v/v) extract, the powdered root, leaf and stem (100 g) was macerated in 1000 ml solvents for 72 h and subsequently the mixture was filtered and concentrated under reduced pressure at 35°C. The yields of extracts are shown in Table 1.

Wood vinegar

The wood vinegar was obtained from the Shimi Pazhouhan Industry Incorporation, Ardabil, Iran. Its quality is determined to be pH (below 3), acid amount (5.6~6.9%), and tar (below 4.0).

Table 1. The yield (% w/w) of *S. leriifolia* extracts.

Tissue collected	Aqueous	Methanol	Ethanol
Root	5	6.8	7.5
Leaf	8.5	5.7	7.4
Stem	6.5	7.5	6

Bioassay

The wood vinegar was prepared at 1, 10, 30, 50 and 100% dilution with water. Only extract 100% of each plant extract was set up for each treatment. The mixture solutions of each plant extracts and wood vinegar were prepared as follows. Half–recommended (50%) of plant extracts were mixed with 30, 50 and 100% dilutions of the wood vinegar. The solution of the wood vinegar, plant extracts or the mixtures were applied to filter papers (Whatman N° 1, cut into 4 × 5 cm paper strip). The impregnated filter papers were put into 1000 mL glass bottles. Twenty adults of *L. serricorne* (7–14 days old) were placed in small plastic tubes (3.5 cm diameter and 5 cm height) with open ends covered with cloth mesh. The tubes were hung at the geometrical centre of glass bottles, which were then sealed with air–tight lids. In the control bottles, water was applied on the filter papers. The exposure times of 24, 48, and 72 h were used in all treatments. There were four replicates for each dose. A complete set of controls were maintained and each treatment was replicated four times. When no leg or antennal movements were observed, insect was considered dead.

Statistical analysis

The percentage mortality was determined for analysis of variance (ANOVA) according to the general linear model (GLM) using the SPSS software version 16.0 for Windows statistical program analysis. Comparison of means was done through Tukey (HSD) test at the 5% level (α = 0.05).

Results

Effect of wood vinegar on L. serricorne

Mortality of water–treated *L. serricorne* was less than 15%. In addition, its rate was not significantly changed by the treatments with various dilutions of the wood vinegar solution and exposure time (Fig. 1).

Effect of wood vinegar mixed with aqueous extracts

Treatments with recommended doses (100%) of root, leaf and stem extracts showed mortality of 91.25, 100, and 71.25% at 72 h, respectively (B). The effects of treatment with 50% dilutions of recommended doses of extracts were lower relative to those of recommended doses (C). Furthermore, extracts (50%) mixed with various dilutions of wood vinegar antagonistic effect on the mortality of *L. serricorne* (Fig. 2).

Effect of wood vinegar mixed with methanol extracts

Treatments with recommended doses (100%) of root, leaf and stem showed mortality of 58.75, 60, and 50% (B). The effects of treatment with 50% dilutions of recommended doses of extract were lower with those of recommended doses. Furthermore, root, leaf and stem extracts (50%) mixed with various dilutions of wood vinegar did synergistic effect on the mortality of *L. serricorne* (Fig. 3).

Effect of wood vinegar mixed with ethanol extracts

Treatments with recommended doses (100%) of root, leaf, and stem showed mortality of 66.25, 71.25, and 60% (B). The effects of treatment with 50% dilutions of recommended doses of extract were lower when compared with those of recommended doses. Furthermore, extracts (50%) mixed with various dilutions of wood vinegar did antagonistic effect on the mortality of *L. serricorne* (Fig. 4).

Effect of plant extracts as single compound on L. serricorne

Treatments with recommended doses (100%) of aqueous, methanol, and ethanol extract showed mortality against insect (B). Aqueous extracts was more toxic than other extracts on insect (Figs 2, 3 & 4).

Discussion

Control of *L. serricorne* populations around the world is primarily dependent upon continued applications of phosphine (PH_3) (White and Leesch 1995, Kim et al. 2003). Little work has been done to manage *L. serricorne* by using aromatic medicinal plants (Namba 1993, Kim et al. 2003). Toxicity of extracts from different *Salvia* species reported against insect pests (Pavela 2004, Gokce et al. 2006). However, there is no published report on the insecticidal activity of *S. leriifolia* extracts against *L. serricorne*. From the standpoint of mixtures of plant extracts and wood vinegar, there is only one study that was done (Pangnakorn et al. 2011); however no report

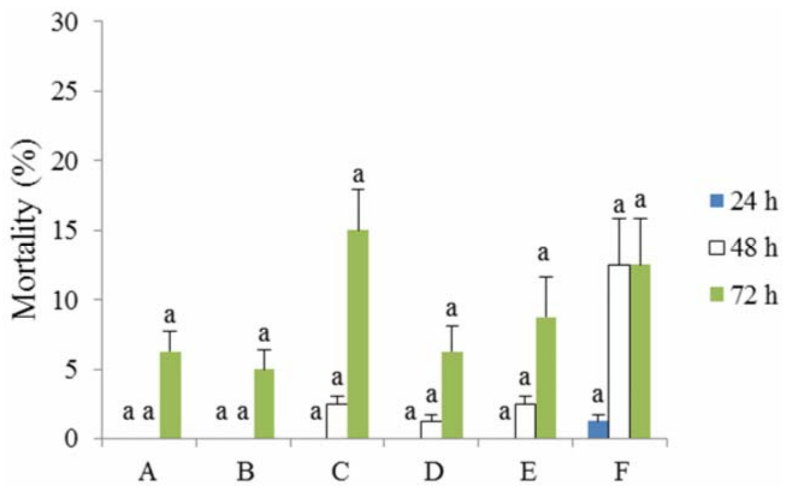

Figure 1. Effects of wood vinegar on the mortality of *L. serricorne*. Wood vinegar was diluted at 1, 10, 30, 50 and 100%. *Lasioderma serricorne* were treated as follows: water–treated (A), 1, 10, 30, 50 and 100% wood vinegar (B, C, D, E and F, respectively).

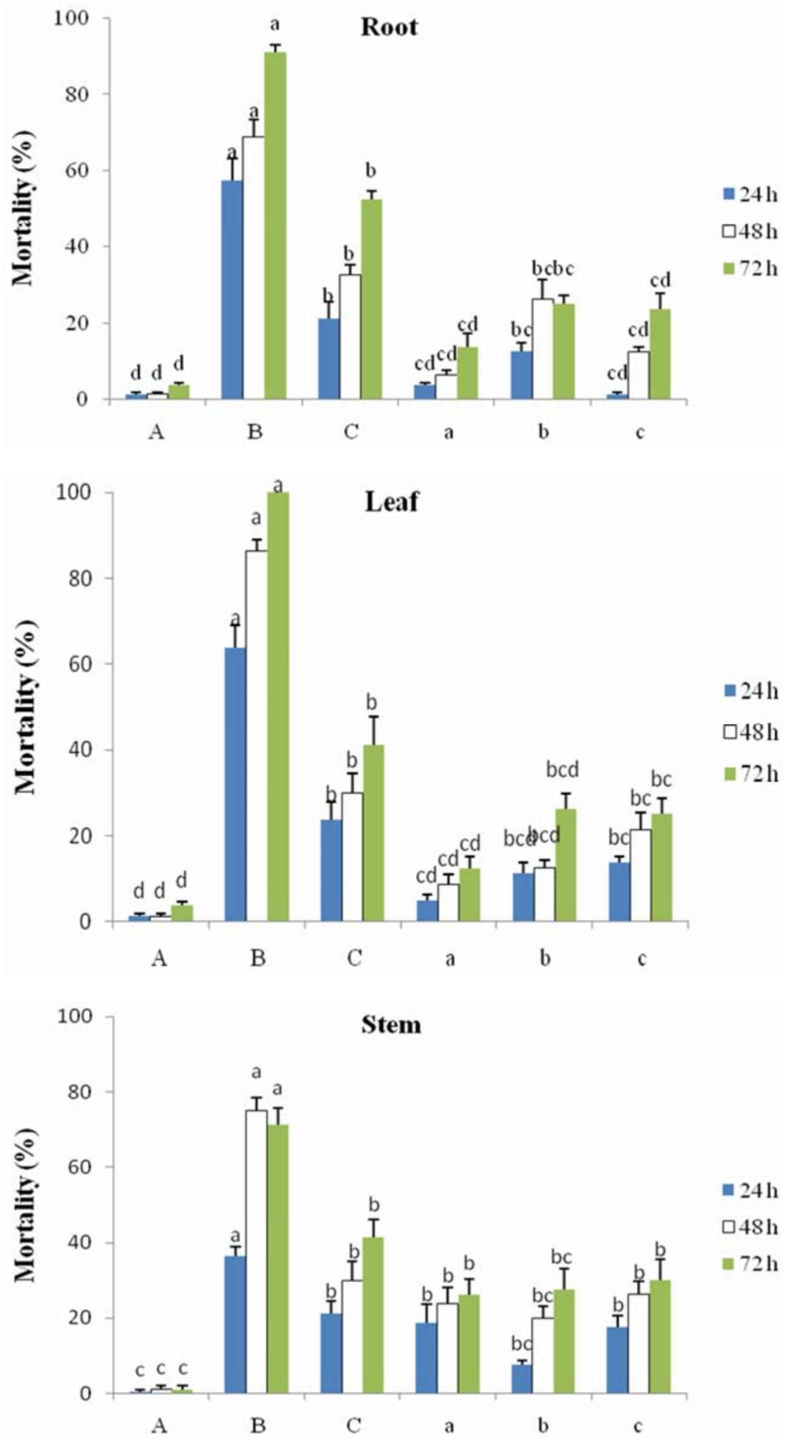

Figure 2. Effects of aqueous extracts and their mixtures with various doses of wood vinegar on the mortality of *L. serricorne*. 50%–diluted extracts were used to make mixture solutions with wood vinegar. *Lasioderma serricorne* were treated as follows: water–treated control (A), 100% extracts only (B), diluted extracts only (50%) (C), and mixtures of diluted extracts (50%) with 30, 50 and 100% wood vinegar (a, b and c, respectively).

was found in related literature on the effects of mixtures on stored product insects. In this respect, this is the first report on the toxicity of *S. leriifolia* as a single compound and in mixtures with wood vinegar that provides some information on the possible interactions between the plant extracts and wood vinegar.

Wood vinegar itself did not show insecticidal effect on cigarette beetle. Thus, we focused on finding alternative role(s) of wood vinegar in the mixture with *S. leriifolia* extracts. Because, plant extracts contain compounds that show toxic effect in insects (Isman 2006). When plant extracts were compared as mixtures possessing two compounds, their toxicity in some cases was different from their toxicity as single compounds. In treatment with mixtures of wood vinegar and extracts only the mixture with methanol extracts had enhanced effect on cigarette beetle while the mixtures with other extracts did not (Fig. 3). This result suggests that wood vinegar has a potential to increase the activity of plant extracts, particularly in the case of methanol extracts. In addition, its effect might be specific to a certain chemical characteristics because other of the extracts tested did not show an increased effect when mixed with wood vinegar.

One of the most valued properties of plant extracts is their fumigant activity against insects, since it may involve their successful use to control pests in storage without having to apply the compound directly to the insects. Based on

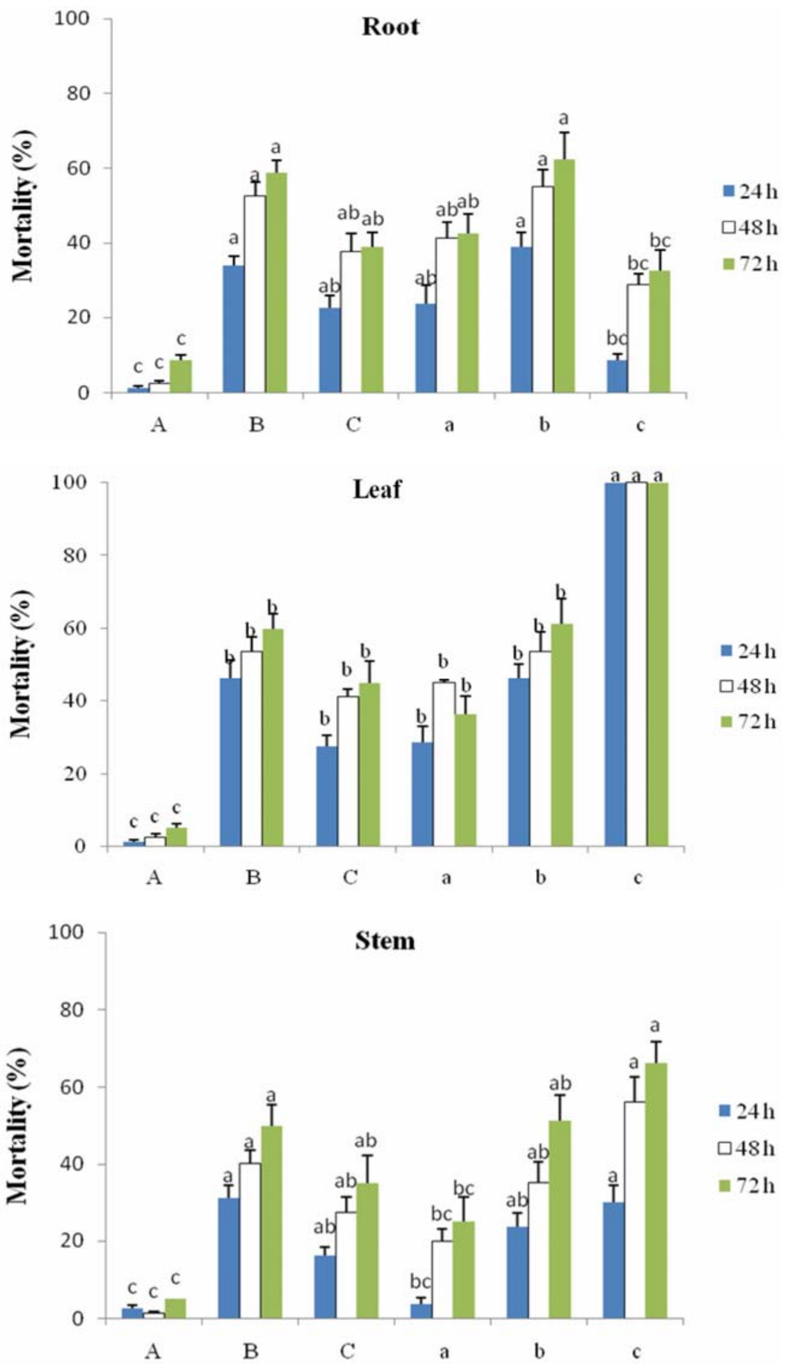

Figure 3. Effects of methanol extracts and their mixtures with various doses of wood vinegar on the mortality of *L. serricorne*. 50%–diluted extracts were used to make mixture solutions with wood vinegar. *Lasioderma serricorne* were treated as follows: water–treated control (A), 100% extracts only (B), diluted extracts only (50%) (C), and mixtures of diluted extracts (50%) with 30, 50 and 100% wood vinegar (a, b and c, respectively).

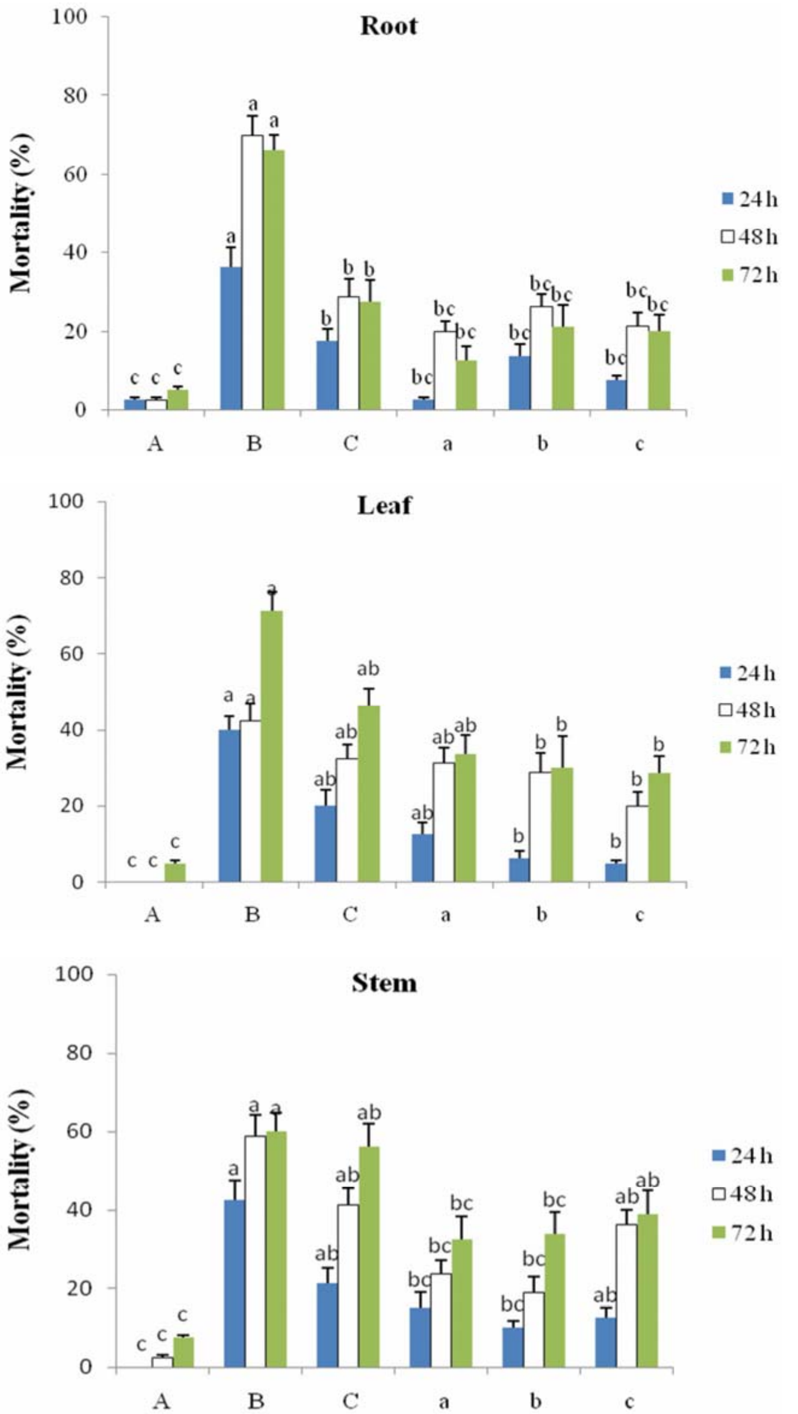

Figure 4. Effects of ethanol extracts and their mixtures with various doses of wood vinegar on the mortality of *L. serricorne*. 50%–diluted extracts were used to make mixture solutions with wood vinegar. *Lasioderma serricorne* were treated as follows: water–treated control (A), 100% extracts only (B), diluted extracts only (50%) (C), and mixtures of diluted extracts (50%) with 30, 50 and 100% wood vinegar (a, b and c, respectively).

the results from fumigant bioassays the plant extracts tested showed high toxicity when they were applied as a single compound against insect with insecticidal activity dependent on plant derived material, extraction, and exposure time (Figs 2, 3 & 4). From the standpoint of susceptibility of insect to plant extracts, aqueous extracts was more toxic than other extracts on insect (Fig. 2). When *L. serricorne* were fumigated with extracts, for leaf aqueous extract, 72 h exposure time was necessary to cause 100% mortality, while other experimental extracts did not caused 100% mortality at three different exposure times. When plant extracts were applied in mixtures with wood vinegar, different mortalities were observed. The methanol extracts have been shown synergistic effects. This was conspicuous when insect exposed with leaf methanol extract (50%) and wood vinegar (100%), where 100% of the population died at 24 h (Fig. 3). In the case of aqueous and ethanol extracts, wood vinegar did antagonistic effect on the mortality (Figs 2 & 4). For example, when insect fumigated with ethanolic leaf extract (50%), percentage of mortality at 72 h exposure time was 46.25, while with 100% of wood vinegar and 50% of extract mortality was 28.75% at same time (Fig. 4). Based on the results of plant–derived material, leaf extracts was more toxic than root and stem extracts. Fumigant toxicity of 100% aqueous, methanolic, and ethanolic leaf extracts was 100, 60, and 71.25%, respectively, at 72 h exposure time. While for root extracts was 91.25, 58.75, and 66.25% and for stem extracts were 71.25, 50, and 60% of aqueous, methanolic, and ethanolic extracts, respectively, at same exposure time.

The solvents used in extracting plant materials for insecticidal potency is highly important as our present study shows. Methanol and ethanol extracts were less toxic than the aqueous extracts. This could be because the active principles in the test plant materials are more soluble in water. It could be demonstrated that the higher efficacy of aqueous extracts over that of methanol and ethanol is because biological compounds present in the Lamiaceae family are readily obtained by distillation with water. Our results in the present study show that the effectiveness of a natural plant extracts increase with decreasing polarity of the solvents used for extraction (Figs 2, 3 & 4).

Wood vinegar has no fumigant toxicity against *L. serricorne* (Fig. 1). The present results are in agreement with previous reports, especially the results presented by Kim et al (2008). In another study by Pangnakorn et al (2011), the toxicity test of wood vinegar on the *Culex quinquefasciatus* (Say) evaluated under laboratory condition by using contact and stomach application. The tests revealed that toxicity of wood vinegar in stomach poison was higher than contact poison. In conclusion, fumigant application of wood vinegar was lower than contact and stomach method. It has could be to chemical components. For example, wood vinegar might enhance the penetration of carbosulfan into the planthoppers because it contains a large amount of acetic acid that may influence the permeability of cuticle layer (Kim et al. 2008). Ethyl valerate is the other main compound of wood vinegar (Yoshimura and Hayakawa 1991). Chaskopoulou et al (2009) reported toxicity of 31 compounds against adult females of *Aedes aegypti* and *Culex quinquefasciatus*. The test revealed ethyl valerate had low vapor toxicity.

It is important to consider that these data are not enough to derive an exact conclusion on the effect of single compounds on each other in mixtures. In order to understand the interaction between different compounds it is probably better to analyze the compounds and find some information about the chemistry of single compounds and possible chemical reactions between compounds when they are mixed. It is possible that the chemical structure of compounds changes during interaction with other compounds and produce metabolites which are more or less toxic than original compounds (Stankovic et al. 2004, Denloye et al. 2006, Alyokhin et al. 2007, Baker et al. 2007, Safaei Khorram et al. 2011). Further study is required to determine the mechanism of synergistic (additive) effect of wood vinegar on the methanol extracts action. In addition, since wood vinegar is a mixture of numerous organic compounds, key molecule(s) that elicit synergistic effect with insecticides should be identified.

In conclusion, the development of natural insecticides will help decrease the negative effects of synthetic chemicals. In this respect, natural insecticides may also be effective, selective, easily biodegradable and relatively low pollution for environment. In the present study, the majority of the compounds examined as single compounds or mixtures were found to be toxic against *L. serricorne*. Therefore, in the light of the present results, it can be suggested that these compounds can be used as new insecticidal reagents against *L. serricorne*.

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