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Identification of Suitable Solvent for Extraction of Principle Insecticidal compounds from Selected Medicinal and Aromatic Plants and Evaluation of their Efficacy against *Plutella xylostella* (L.)

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ABSTRACT: A laboratory experiment was carried out to identify a suitable solvent for extraction of principle insecticidal compounds from selected medicinal and aromatic plants. Further, these insecticidal compounds were evaluated for their efficacy against diamondback moth, *Plutella xylostella* (L.). Four different solvents such as methanol, chloroform, ethyl acetate and water were used to extract insecticidal compounds from the four plants namely, neem, pongemia, datura and bael. Based on the LC_{50} and LT_{50} values registered with four solvents and four plants, the order of suitability of solvent in extracting the insecticidal principle compounds from the plants is as follows; chloroform > aqueous > methanol > ethyl acetate. When the different plants are considered for their efficacy irrespective of the solvents used for extraction of insecticidal principle compounds from them, the order of effectiveness is as follows; neem > datura>bael>pongamia.

Keywords: DBM, solvents, LC_{50}, LT_{50}, medicinal and aromatic plants extract, bioassay, Soxhlet's extractor and magnetic stirrer.

INTRODUCTION

The diamondback moth (DBM)is the most destructive and dreaded pest on cruciferous vegetables causing up to 90 per cent loss in the marketable yield of cabbage (Gashawbeza, 2006). The damage is caused by larva which skeletonizes the foliage of host plant and renders it unfit for consumption. Till date, at farmer's level, insecticides have been the primary means to manage this pest. But the control achieved in many cases is unsatisfactory as pest has a capacity to develop resistance rapidly to almost all insecticides applied in the field, including bio-pesticides such as *Bt.* and spinosyns,besides causing inherited problems such as resurgence, secondary pest outbreak, environmental pollution etc. (Tabashnik *et al*., 2013). Now, it has been very difficult to control this increasing pest population even with the newer molecules of insecticides. With this background, an effort was made to exploreuse of medicinal and aromatic plants which are having insecticidal properties in managing this noxious pest. These plants are getting popularity in recent years among the farmers particularly with organic growers owing to their safety and slow rate of resistance development (Liang *et al*., 2003).

MATERIALS AND METHODS

The different solvents such as methanol, chloroform and ethyl-acetate, and water were used in the current research to find out most suitable solvent for extracting insecticide principle compounds from neem, pongamia, datura and bael plants.

Collection of plant materials: The selection of medicinal and aromatic plants used in the present study was made on the basis of their known or unknown efficacy against insect pests from a thorough review and also their availability. The plants such as neem, pongemia, datura and bale were collected from the University of Agriculture, Sciences, GKVK campus and medicinal block of College of Horticulture, Bengaluru (Karnataka: India).

Mass rearing of the test insect, *Plutella xylostella:* The culture of diamondback moth was maintained in the laboratory by adopting the method described by Liu and Sun (1984) with suitable modifications. The mustard seedlings were raised in the plastic cups (8×4 cm). The plastic cups were filled with well soaked vermiculite to a depth of 1.5 cm was used as a growth medium. Then the bold seeded mustard seeds were sown uniformly over the vermiculite medium surface and watered. The seeds were germinated within three to four days at room temperature and the seedlings were watered as and when required.

The DBM culture was initiated in the laboratory by collecting the late instar larvae and pupae from cabbage fields in and around Bengaluru. The larvae were reared on mustard seedlings raised in the laboratory up to pupal stage and pupae were collected with the help of fine forceps. Then, such collected pupae were placed in the oviposition cage (35 cm × 10 cm × 35 cm) for adult

emergence. Ten per cent honey syrup on cotton wad was provided as food for adults during oviposition. One day old moths were provided with three to four days old mustard seedlings in the oviposition cage to facilitate oviposition by moths. After 24 hours, seedlings only with eggs were transferred to rearing cages. Fresh seedlings were provided for oviposition every day.

The hatched neonate larvae were fed on mustard leaves by mining, and later instar larvae fed on entire leaves. When the seedlings had been largely consumed, the larvae were transferred to fresh seedlings by gently tapping the seedlings with the help of a camel hairbrush and continued till the larva attain to the pupal stage. When the larvae were in fourth instar, folded papers were provided in the rearing cages to facilitate pupation. Whenever large numbers of uniform age group larvae were required for the studies, the pupae were stored in the refrigerator for a few days to synchronize the moth emergence. Only F_1 generation larvae were used in the laboratory studies.

The rearing trays and cages were disinfected regularly with two per cent sodium hypochlorite to prevent fungal and bacterial infections. Care was taken to keep away ants, lizards and spiders.

Preparation of plant extracts: The parts of selected plants were washed thoroughly with water and shade dried for a one week and then ground to powder by an electrical grinder. The principle compounds were extracted from the selected plants by using different solvent by adopting standard extraction methods followed by Mishra and Singh (2014); Amerasan *et al*. (2015), respectively.

Aqueous extracts. The aqueous extract of selected plants was prepared by adopting the method followed by Mishra and Singh (2014). 10g of powdered sample was stirred with 100 ml of water by magnetic stirrer for eight hours. Then the material was filtered through a Whatman No. 1 filter paper in a conical flask. The filtered extract was kept in a hot water bath at 60°C temperature till complete evaporation of water. The weight of thus obtained semisolid crude extract was taken and finally prepared as a stock solution by adding known quantity of water and used for further bioassay studies.

Methanol, Chloroform and Ethyl acetate extracts: The extraction method adopted by Amerasan *et al*. (2015) was followed for preparing methanol chloroform and ethyl acetate extracts of selected plants. 4g of powdered sample was taken in thimble and 80 ml of respective solvent was taken in a flask and loaded to the Soxhlet's extractor. Sample was kept at 60°C temperature for one hour and remaining solvent evaporation was done at a boiling temperature for one and half an hour. Finally, the obtained crude extract was weighed and prepared as stock solution by adding known quantity of water and used further bioassay studies.

Bio-assay method: The insecticidal activity of selected plants was assessed by conducting bioassays by adopting a standard leaf dip bioassay method (Kumar *et al.,* 2000). Freshly moulted early third instar DBM larvae were used as test insects for all the bioassays.

The concentrations of respective plants were prepared from the stock solution by serial dilution. For seed and leaf plants extracts, the concentrations maintained were 2.5, 2.0, 1.5, 1.0 and 0.5 per cent concentrations. Soap solution at the rate of 0.05 per cent was used in all the treatments and control as sticking agent. The uniform sized leaf discs were prepared from fresh cabbage leaves. These discs were dipped in the solutions of predetermined concentrations of respective plant extracts for 15 seconds and air dried in shade and transferred to Petri plate. Ten freshly hatched early third instar larvae of DBM were released on the treated leaves and served as a replication. Three such replications were maintained for each concentration. Along with the treatments, larvae treated with distilled water served as control. The Petri plates were kept at room temperature. The treated leaves were withered in about 48 hours. Therefore, such leaves were replaced with fresh but untreated leaves. Leaf changes were continued till the complete larval mortality or pupation of all test insects.

RESULTS AND DISCUSSION

In the present investigation, four solvents *viz.*, methanol, chloroform, ethyl acetate and aqueous were used to identify most suitable solvent to extract maximum amount of principal insecticidal compounds from four medicinal and aromatic plants. The efficacy of such plant extracts was assessed by adopting leaf dip bio- assay method under laboratory conditions and the data was subjected to the probit analysis and expressed as LC_{50} and LT_{50}.

The present study results revealed that, among all the four plant extracts tested with different solvents, the chloroform extract of neem seed kernels showed highest toxicity by registering lowest LC_{50} value of 0.52 per cent, followed by pongamia (1.00%), datura (1.03%) and bael leaf extracts (1.14 %). The Aqueous extracts of neem stands second position and showed higher toxicity with LC_{50} value of 0.54 per cent. In the case of bael, datura and pongamia, the LC_{50} values were 1.02 1.09 and 1.14 per cent, respectively. Whereas, the Ethyl acetate extract of neem seed kernels showed lesser toxicity with higher LC_{50} of 0.95 per cent followed by bael leaf (0.96%), datura leaf (1.32%) and pongamia leaf extracts (1.45%) (Table 1).

The LT_{50} values of all the tested four solvents with different plants indicated that, the chloroform extract of neem seed kernels found to be more toxic by registering lowest LT_{50} with 26.54 hours followed by pongemia (29.56 hrs.), bael (38.68) and datura (43.09) at 2.5 per cent concentration. The methanol extract of neem seed kernel was next best solvent with LT_{50} value of 32.78 hours followed by pongamia, bael and datura with 36.62, 49.04, and 53.38 hours, respectively at same concentration. The highest LT_{50} values were recorded with aqueous extracts of pongamia (45.87 hours) followed by neem (46.68 hrs), datura (63.30 hrs) and bael (69.34 hrs) (Table 2).

On the whole, based on the LC_{50} and LT_{50} values registered with four solvents and four plants, the order of suitability of solvent in extracting most of the

insecticidal principle compounds from the four plants is as follows; chloroform > aqueous > methanol > ethyl acetate (Fig. 1). When the different plants are considered for their efficacy irrespective of the solvents used for extraction of insecticidal principle compounds from them, the order of effectiveness is as follows; neem > datura>bael>pongamia. However, when all the plant extracts compared for their lethal time, the seed ectract of pongamia emerged as superior plant by registering lowest LT 50 value, followed by NSKE, datura and bael (Fig. 2).

The present findings are in conformity with that of Lingaturai *et al*. (2010), who reported that, among hexane, ethyl acetate and chloroform extract tested with *Acalypha fruticosa*, the chloroform extract showed maximum larvicidal activities at 5 per cent concentration with a LC_{50} value of 1.86 per cent against third instar larvae of DBM. Similar findings were also obtained by Jawalkar *et al*. (2016) where Soxhlet's extracted datura seed extract in ethanol, chloroform and acetone found to be effective against diamond back moth, while extracts in methanol and n-hexane showed poor results. The present findings are also in line with that of Bakavathiappan *et al*. (2012) where maximum toxicity was noticed with chloroform extract of calotropis against the *Spodoptera litura* with a LC_{50} value of 2.854 followed by acetone (6.65), ethyl acetate (8.748) and methanol (28.45). Further, the chloroform extract exhibited the best larvicidal activity (67%). In the present investigation, the least toxicity was obtained with those plants where ethyl acetate was used for extraction as compared to other solvents used. Similar findings were also obtained by Matharu and Mehta (2016), where ethyl acetate extract of *Acorus calamus* registered only about 36.54 per cent at 5 per cent concentration.

Table 1: The lethal concentration (LC_{50}) of four plant extracts against early third instar larvae of *Plutella xylostella* at 120 h after treatment.

Solvent Extracts	LC_{50}(%)			
	Neem seed kernel extract	Pongamia seed extract	Leaf extract of Bael	Leaf extract of datura
Aqueous	0.54	1.14	1.02	1.09
Methanol	0.56	1.11	1.32	0.99
Chloroform	0.52	1.00	1.14	1.03
Ethyl acetate	0.95	1.45	0.96	1.32

Table 2: The lethal time (LT_{50}) of four plant extracts against early third instar larvae of *Plutella xylostella* at 2.5 concentration.

Solvent Extracts	LT_{50} (h)values			
	Neem seed kernel extract	Pongamia seed extract	Leaf extract of Bael	Leaf extract of datura
Aqueous	46.67	45.87	69.35	63.30
Methanol	32.77	36.62	49.04	53.38
Chloroform	26.53	29.56	38.68	43.09
Ethyl acetate	35.34	31.16	58.92	52.05

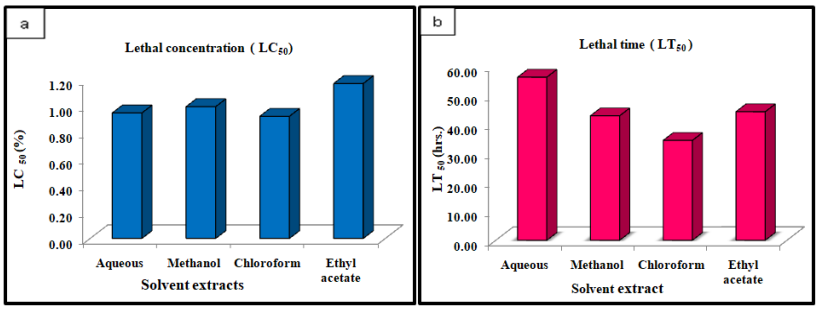

Fig. 1. The pooled mean of lethal concentration (LC_{50}) and lethal time (LT_{50}) of four different solvent extracts of different plants against early third instar larvae of *Plutella xylostella*.

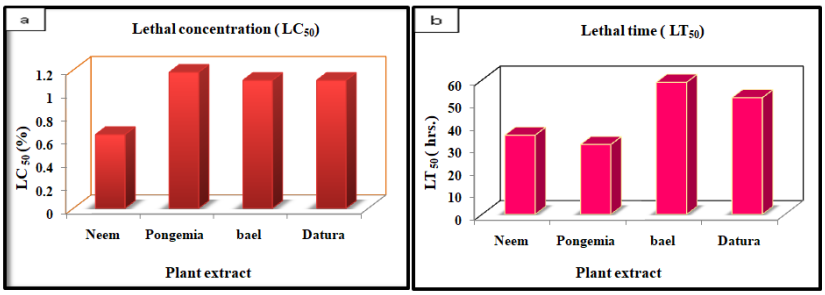

Fig. 2. Pooled mean of lethal concentration (LC_{50}) and lethal time (LT_{50}) of four plant extracts with different solvents against early third instar larvae of *Plutella xylostella.*

CONCLUSIONS

Among the different solvents such as methanol, ethyl acetate, chloroform and water evaluated for their suitability in extracting maximum amount insecticidal compounds from test plants, the chloroform extract of neem seed kernels showed highest toxicity by registering lowest LC50 and LT50 values against early third instar larvae. Next best plants in the order of toxicity were seed extract of pongamia, leaf extract of datura and bael.

It can be concluded from the present investigation that, the chloroform extract found more suitable solvent in extracting maximum amount of insecticidal compounds from the medicinal and aromatic plants as that of other solvents tested.

FUTURE SCOPE

— Principle insecticidal compounds of the plants which exhibited superior efficacy in the present study needs their proper identification for further evaluation.

— The combinations of different medicinal and aromatic plants need to be evaluated for their compatibility in respect of enhancing the insecticidal activity.

Conflict of Interest. None.

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