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Screening of some weeds for larvicidal activity against *Aedes albopictus*, a vector of dengue and chikungunya

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ABSTRACT

Background & objectives: Screening of crude extracts of plants facilitates the establishment of highly effective extract for mosquito control. This practice should be preferred before in depth study of plant extracts rather than spending much efforts and energy in detailed examinations for practically ineffective extracts. In this study, leaf powders of four weed plants were used for the quick screening of effective plant extract as larvicide against III instar larvae of *Aedes albopictus* Skuse. At the same time, effect of different seasons on the larvicidal efficacy of plants and selection of proper solvents for further investigation were also studied.

Methods: Leaves of *Vernonia cinerea*, *Prosopis juliflora*, *Hyptis suaveolens* and *Malvastrum coromandelianum* plants were collected in summer, winter and rainy seasons from Madhya Pradesh region (India). To assess the larvicidal efficacy the suspensions of leaf powders in different solvents (isopropanol, methanol, acetone, dimethylsulfoxide and water) were used for larvicidal bioassay. The mortality counts were made after 24 h and the LC₅₀ and LC₉₀ values were calculated.

Results: Results showed that leaf powder of *V. cinerea* in acetone collected during summer showed highest efficacy with LC₅₀ value of 0.22 g/l and LC₉₀ of 0.96 g/l followed by methanolic solution of *P. juliflora* with LC₅₀ of 0.44 g/l and LC₉₀ value as 1.85 g/l. Amongst all solvents, leaf powder in acetone; while among seasons, summer collected plant materials were found to be more effective larvicides as compared to others.

Interpretation & conclusion: Summer and winter collected leaves of *V. cinerea* and *P. juliflora* dissolved in the solvents of medium polarity range showed significant larval toxicity and thus suggests a detailed study on these plants as potential larval control agents.

Key words *Aedes albopictus*; larvicide; leaf powder; seasonal variation; weeds

INTRODUCTION

Aedes albopictus (Skuse) (Diptera: Culicidae) is a day biting mosquito which transmits many arboviruses and causes dengue and chikungunya¹⁻². One of the methods to manage these diseases is to control the vectors, and cause interruption in the disease transmission. Use of synthetic insecticides to control mosquitoes is very common due to their quick action, but their continuous use may lead to the development of resistance and permanent residual effect on the non target organisms, including human³. These factors create the need to develop easily biodegradable and effective alternative insecticides.

Various plant derivatives have been reported for their insecticidal activity against mosquitoes. Plant products are easily degradable, less hazardous and rich reservoir of chemicals, having diverse biological activity for vector control, hence the use of plant products is becoming more popular as compared to synthetic insecticides⁴.

For the exploration of effective larvicide against

mosquitoes the preliminary screening of crude botanical extract of different plants is essential to determine the general potency. Toxic effect of plant extracts vary with plant species, mosquito species, geographical location and plant parts, extraction methodology and polarity of solvents used, hence it is important to select suitable plant.

It has been seen in various literatures that most of the studies on the larvicidal efficacy of plant products focused on well known horticultural, medicinal and other plants of economic importance. The extensive use of plants of economic importance for commercial purpose can make the plants endangered and causing threat to ecosystem. Therefore, selection of weeds as botanical larvicide against mosquitoes has several advantages, as these are easily available and require little technical input and time for cultivation and procurement. Hence, the present study is focused on screening of four locally available weed plants for their potential larvicidal activity.

Vernonia cinerea (Asteraceae) commonly known as

'Little Iron' weeds and 'Sahdevi' in Hindi, is an annual weed widely distributed in India. This is a well known medicinal plant and used in indigenous medicine for cold, asthma and bronchitis⁵. *V. cinerea* has also been reported for insect antifeedant property⁶.

Prosopis juliflora (Fabaceae) commonly known as 'Vilayati Babul' in Hindi, is a drought resistant and exotic weed of semi arid areas of India. Leaf extract of this plant was found to be toxic against various microbes⁷. Singh and Saratchandra⁸ stated that this plant also has some insecticidal property.

'Bush Tea' (*Hyptis suaveolens*) belongs to the family Lamiaceae and is a native of tropical America. This aggressive annual weed is wide spread in India. Essential oil of this plant has been reported to have some insecticidal and larvicidal properties. It was also found to have repellent activity against adult *An. gambiae* mosquito⁹.

Malvastrum coromandelianum from Malvaceae family, a weed locally known as 'Kharenti' (False mallow) has been reported for its antimicrobial property¹⁰.

Most of the earlier studies were focused on detailed investigation of those plant products, which were not much effective as mosquito larvicides. In the present study, a methodology has been adopted which may help in the establishment of highly effective crude botanical extract in first level of screening, rather than going for large extraction processes and consuming large amount of plant materials. Definite amount of the leaf powder was dissolved in 1 ml of solvents of different polarities and this solution was used for larval bioassay. It is useful in the quick screening and selection of proper solvent, which extracts most of the potentially active constituents from the plant material for further investigation. However this method can be applicable to nontoxic solvents only. Therefore, the present study was carried out with an aim to determine the larvicidal efficacy of commonly available weeds by using their leaf powder.

MATERIAL & METHODS

Plant material

The green leaves of four plant species—*V. cinerea*, *P. juliflora*, *H. suaveolens* and *M. coromandelianum*, were collected in summer (April 2012), winter (December 2012) and rainy season (August 2012) from district Gwalior, Madhya Pradesh, India. Each specimen was identified with the help of expert from School of Studies in Botany, Jiwaji University, Gwalior, India and voucher specimens were preserved in the laboratory of Defence Research and Development Establishment (DRDE),

Gwalior. Plant material was dried under shade for 15 days.

Preparation of experimental doses

The leaves were powdered to fine particles of mesh size 250 μm approximately. Powdered material was weighed in 0.2, 0.4, 0.6, 0.8, 1 g quantity and soaked in 1 ml of each solvent; isopropanol, methanol, acetone, dimethyl-sulfoxide and water (in separate vials for each concentration and replication), at 25°C with occasional shaking. After 24 h, the entire 1 ml solution in each vial was used as such (without filtering) for larval bioassay to get the final concentration of leaf powder as 2, 4, 6, 8, 10 g/l in water.

Mosquitoes

Larvae were taken from cyclic colony of *Ae. albopictus* mosquito maintained in DRDE laboratory under standard rearing conditions of 27±2°C room temperature and 70±10% relative humidity. Larvae were kept in bowls (2.5 l) and definite amount of yeast powder was provided as larval food. Adult mosquitoes were reared in wooden cages (75 × 65 × 65 cm) and 10% sugar solution *ad libitum* dispensed through a cotton wick in a Petri dish were provided as food. Moist filter paper was kept in a beaker inside the cage for collecting eggs. Females were offered rabbit blood once in a week.

Larval bioassay

Early III instar larvae of *Ae. albopictus* were used for larvicidal bioassay as per WHO procedure with some modifications¹¹. Experiments were carried out in four batches with control. A total of 20 larvae were released in glass beakers (250 ml) containing 99 ml tap water. Different doses of leaf powders in solvents (1 ml) were added in each beaker containing larvae and the controls were treated with 1 ml of solvents. Larval mortality was recorded after 24 h of the treatment.

Data analysis

Larvicidal activity and the seasonal factors, which influence the bioefficacy of plants against *Ae. albopictus* larvae, were assessed in the present study. Mean value of each response variable was calculated (Descriptive analysis, Sigma Stats 3.0). To determine the lethal concentrations (LC₅₀ and LC₉₀ values) for each plant species, data were analyzed by Probit analysis using POLO PC software¹². Analysis of variance (ANOVA) was used to measure the effects of various factors which influence the larvicidal efficacy of plant such as concentration of plant material, solvents used and the seasonal collection of plant material (Sigma Stats 3.0). To summarize the effect of

each studied factors, data were compared in linear model using least square (LS) means. Differences in LS means of the dependent variables for each density were examined by performing Tukey test for pair wise comparisons.

RESULTS

The variation in the larval toxicity of four weeds collected in different seasons is shown in Table 1. The LC₅₀ and LC₉₀ values for different plants varied with concentration of suspension, solvents and with the seasons in which the plant leaves were collected. The results revealed that for each plant, summer collected leaves were more toxic to *Ae. albopictus* larvae as compared to the leaves collected in winter and rainy seasons. Dose dependent response in larval mortality was also noticed for each plant species. Dry leaf powder dissolved in acetone and methanol were found to be more effective as compared to other solvents. Among all the plant species, summer collected leaf powder of *V. cinerea* in acetone showed highest efficacy with LC₅₀ value as 0.22 g/l and LC₉₀ as 0.96 g/l followed by methanolic solution of *P. juliflora* with LC₅₀ as 0.44 g/l and LC₉₀ value as 1.85 g/l.

Table 1. LC₅₀ and LC₉₀ values of four plant extracts in different solvents with seasonal variation in toxicity against *Ae. albopictus* larvae

Solvent extract	Summer		Winter		Rainy	
	LC ₅₀ (g/l)	LC ₉₀ (g/l)	LC ₅₀ (g/l)	LC ₉₀ (g/l)	LC ₅₀ (g/l)	LC ₉₀ (g/l)
<i>Vernonia cinerea</i>						
Isopropanol	0.53	2.42	0.54	2.55	1.02	5.95
Methanol	0.51	1.58	0.40	1.97	1.34	5.51
Acetone	0.22	0.96	0.32	1.20	0.98	5.85
DMSO	1.17	5.91	1.12	5.38	1.62	11.69
Water	1.15	4.83	1.05	5.03	1.55	6.63
<i>Prosopis juliflora</i>						
Isopropanol	0.61	1.73	1.07	6.34	0.99	5.59
Methanol	0.44	1.85	0.62	2.18	0.86	4.58
Acetone	0.58	2.23	1.04	4.41	1.06	4.09
DMSO	1.17	5.76	1.12	4.72	1.37	5.26
Water	1.04	3.40	1.49	5.71	1.78	7.44
<i>Hyptis suaveolens</i>						
Isopropanol	0.90	4.50	0.89	4.56	1.09	4.59
Methanol	0.94	4.68	0.79	2.91	1.04	5.28
Acetone	0.82	3.03	0.91	3.40	1.11	6.58
DMSO	1.59	8.99	1.31	6.35	1.55	7.375
Water	1.56	8.21	1.40	5.89	1.55	7.38
<i>Malvastrum coromandelianum</i>						
Isopropanol	1.02	3.44	1.03	4.10	0.97	3.70
Methanol	0.75	2.34	0.84	3.7	0.90	3.77
Acetone	0.62	1.76	0.62	1.91	0.99	4.75
DMSO	0.99	3.36	1.02	3.95	1.68	8.43
Water	1.36	6.39	1.41	7.67	1.53	6.58

Table 2. Three way ANOVA results for the effect of different factors on the toxicity of plant crude extract against *Ae. albopictus* larvae

Plant	Factors analyzed	MS*	F**	p-value
<i>Vernonia cinerea</i>	Seasonal	97.36	108.99	<0.001
	Solvent	73.05	81.79	<0.001
	Concentration	200.88	224.89	<0.001
<i>Prosopis juliflora</i>	Seasonal	59.63	65.90	<0.001
	Solvent	48.92	54.06	<0.001
	Concentration	215.50	238.19	<0.001
<i>Hyptis suaveolens</i>	Seasonal	9.79	13.55	<0.001
	Solvent	39.75	54.99	<0.001
	Concentration	226.04	312.76	<0.001
<i>Malvastrum coromandelianum</i>	Seasonal	20.03	73.03	<0.001
	Solvent	15.70	57.26	<0.001
	Concentration	217.42	792.94	<0.001

*MS—Mean square, **F—F ratio.

Three-way ANOVA demonstrated that the overall efficiency of plant species as larvicide significantly affected with the concentration of leaf powder, solvent used to dissolve the leaf powder and the season of leaves collection (Table. 2). In all four plant species, concentration of dry powder strongly contributed towards the toxicity effect against larvae ($F = 224.89, 238.19, 321.76, 792.94$ for *V. cinerea, P. juliflora, H. suaveolens* and *M. coromandelianum* respectively; $p < 0.001$).

Overall effect of different factors influencing the bioefficacy of plants is summarized in Fig. 1. The data showed that among the plants collected during the three different seasons, plants collected during summer showed more prominent result than other two seasons. Amongst solvents, acetone and methanol suspension exhibited variation in toxicity while isopropanol, DMSO and water dissolved suspension showed comparatively less variations

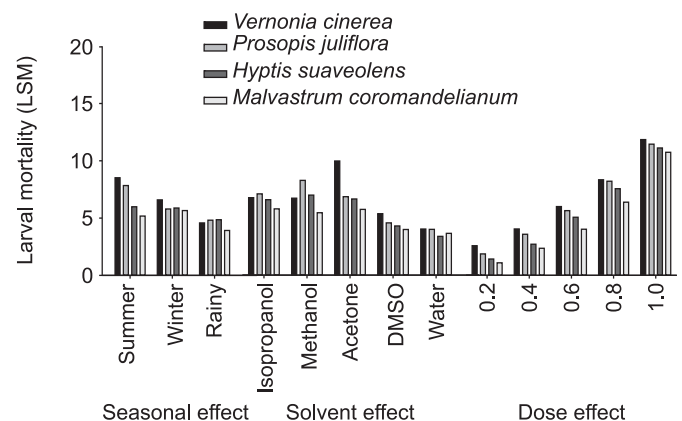


Fig. 1: Least-square means (LSM) for the association of different factors on the larvicidal efficacy of plants.

with respect to dose and plant species. Larval toxicity was strongly influenced with concentration of dry leaf powder and all four plant species exhibited the larval toxicity in order of —*V. cinerea* > *P. juliflora* > *H. suaveolens* > *M. coromandelianum* with increase in concentration.

Fig. 2 represents the overall association of influencing factors with larval mortality caused by different plant species. *V. cinerea* exhibited highest larval mortality in acetone suspension and the mortality increased with increase in concentration (Fig. 2a). Summer and winter

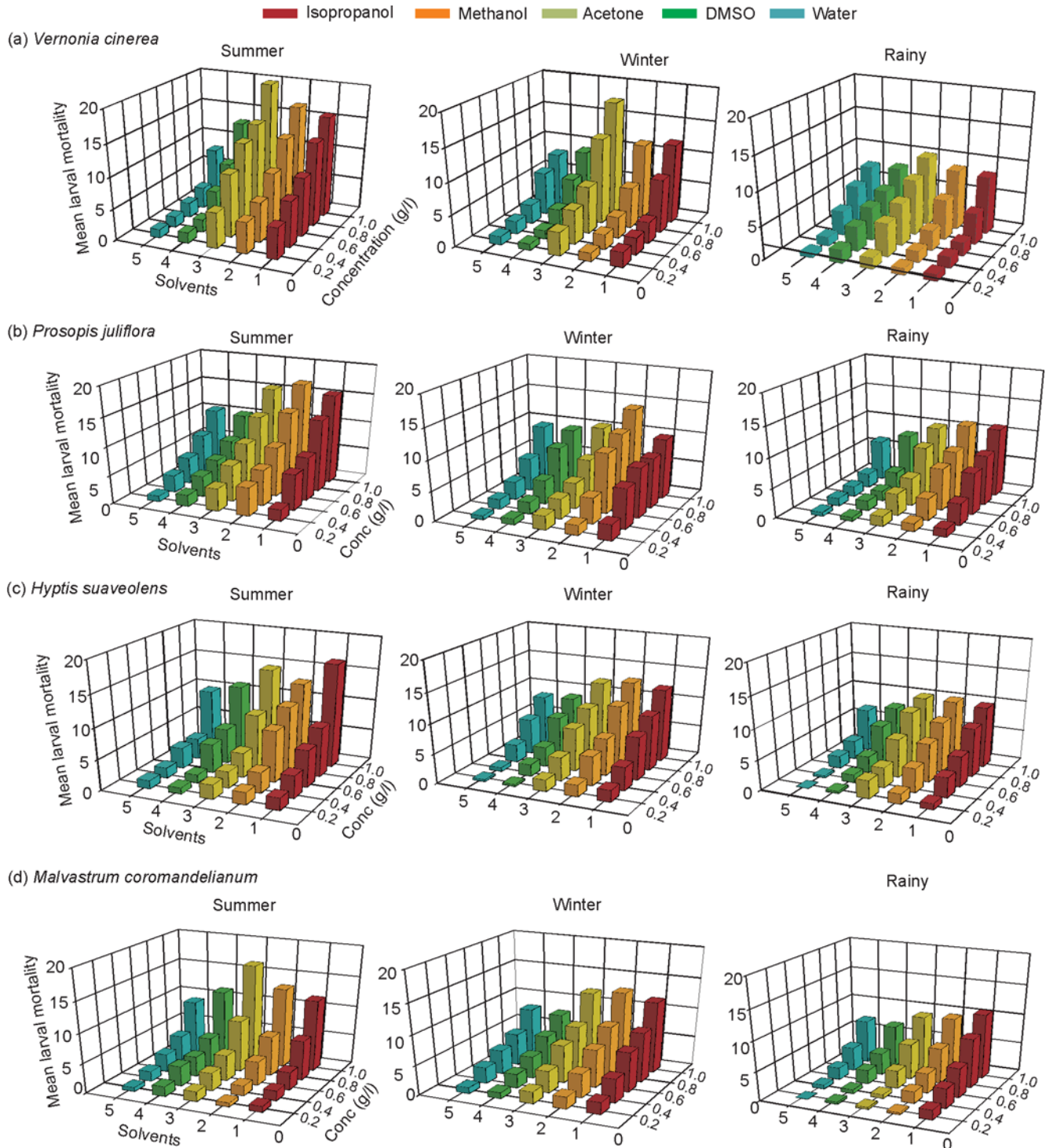


Fig. 2: Comparative study of the effect of solvent extraction, collection of plant in different seasons and concentration of plant extract on the larval toxicity of *Ae. albopictus* (Solvents: 1–Isopropanol, 2–Methanol, 3–Acetone, 4–DMSO, 5–Water).

collected leaves of *V. cinerea* showed significant activity, as compared to rainy season collected plant material, where it showed < 50% (data not shown) mortality at the highest concentration of 1.0 g/l. *P. juliflora* leaf powder in methanol was observed to be more effective and like *V. cinerea*, it also showed maximum activity in summer and winter (Fig. 2b). As shown in Figs. 2 (c) and (d), *H. suaveolens* and *M. coromandelianum* were found to be considerably less effective as compared to *V. cinerea* and *P. juliflora* against *Ae. albopictus* larvae. *H. suaveolens* showed significant larval mortality in isopropanol suspension, whereas *M. coromandelianum* in acetone suspension at highest concentration (1 g/l). For both of the plant species, summer collected plant material showed significant activity while almost similar results were observed in winter and rainy season collected plant materials.

DISCUSSION

The present investigation revealed the possible utilization of some of the selected weeds as mosquitocidal agents against *Ae. albopictus* larvae. The impact of some factors on the larvicidal efficacy of plants such as seasonal variation of plant metabolites, concentration and the solvents to extract plant metabolites were also studied. In the study, leaf powders were used for the quick screening of effective plant as larvicide and selection of proper solvents for further investigation. Very few plants have been studied for their larvicidal efficacy of dried leaf powder. Leaf powder of *Spilanthes mauritiana* and *Piper guineense* in water have been reported to show LD₅₀ values of 0.98 and 0.76 g/l against *An. gambiae* and LD₅₀ values of 0.85 and 0.68 g/l against *Cx. quinquefasciatus* respectively¹³. Work on the dried leaf powder of the weeds is lacking in literatures. To our knowledge, previous larvicidal experiments involved the use of extracts and not crude powder.

In the present screening, leaf powder of *V. cinerea* in acetone was found to be most effective against *Ae. albopictus* larvae. Ethyl acetate extract of *V. cinerea* leaves was found to be effective against *Cx. quinquefasciatus* larvae¹⁴. This plant belongs to the Asteraceae family and various plants of this family like *Chrysanthemum cinerariaefolium*, *Artemisia campestris* and *Tagetes erecta* were reported to have promising larvicidal potential against mosquitoes¹⁵⁻¹⁷. Plants of Asteraceae are known to have various types of phytochemical compounds such as flavonoids, sesquiterpenes, thiophene derivatives and these compounds have been reported to have insecticidal activity.

P. juliflora showed significant larval mortality in

methanolic leaf powder solution. Some studies have also reported its larvicidal potential. Sakhivadivel and Daniel¹⁸ screened variety of plants for larvicidal activity and found that petroleum ether extract of *P. juliflora* showed LC₅₀ in the range of 100-200 mg/l against *Cx. quinquefasciatus* larvae. Recently, Bansal *et al*¹⁹ have also reported the larvicidal potential of methanolic leaf extract of this plant against *An. stephensi* (LC₅₀ = 92.6 mg/l), *Ae. aegypti* (LC₅₀ = 128 mg/l) and *Cx. quinquefasciatus* (LC₅₀ = 118.8 mg/l).

H. suaveolens showed lower mortality as compare to *V. cinerea* and *P. juliflora*. However, it is reported for its repellent property against mosquitoes by Palsson and Jaenson²⁰. In their study, they have shown that *H. suaveolens* provided approximately 84% protection for 2 h against *An. gambiae*. Although, it is good repellent but not showed significant larvicidal activity. Findings of the present study agree with the results of Cavalcanti *et al*²¹ which showed LC₅₀ value of 261 mg/l, however, our results do not correlate with Amusan *et al*²² as their results revealed high mortality rate (80%) in ethanol extract of *H. suaveolens* at conc. 0.9 mg/l against *Ae. aegypti* larvae.

M. coromandelianum showed lowest larval mortality among all the four plant species with LC₅₀ value of 0.62 g/l for acetone solvent; however, it showed delayed growth of larvae. Methanolic leaf extract of this plant has been earlier reported for larvicidal activity against *Cx. quinquefasciatus* larvae²³. The dose dependent response in larval mortality was noticed in the present study. Larvicidal activities of plant powders varied with the plant species, their collection conditions, concentration of plant material and solvents used.

In the present screening, it was observed that the plants which were collected in summer season were noticeably more active than the plants collected in winter and rainy seasons. Secondary metabolites including terpenes, phenolics, and alkaloids defend plants against a variety of herbivores and pathogenic microorganisms as well as various kinds of abiotic stresses. Toxic effects of plants vary depending on the level and type of these secondary metabolites. It is reported in various literatures that quantity and quality of secondary metabolites associated with the environmental stress conditions, which favours the higher concentration of defense chemicals²⁴⁻²⁷. Sahoo *et al*²⁸ evaluated the variations in the total amount of secondary metabolites of some plants during different seasons and found that summer season was most favourable for the production of secondary metabolites.

Among all the solvents used for the plant species, acetone and methanol exhibited remarkable larval mortality as compared to the other solvents. Crude powder in

both of these solvents might have some complex mixture of biocidal active compounds including phenolics, terpenoids, flavonoids and alkaloids which may jointly or independently contribute to produce mortality and delayed growth of larvae. The mode of action and site of effect for larvicidal phytochemicals has received little attention. Ray *et al*²⁹ and David *et al*³⁰ stated that botanical derivatives disrupt the midgut epithelium and adversely affect the gastric tract of mosquito larvae which ultimately causes the larval mortality.

From this screening, two plant species *V. cinerea* and *P. juliflora* were identified as potential larvicide for further detailed study. The results of this study are also useful for the determination of lethal and effective solvent extract as well as the environmental conditions for plant collection in order to select better plant-derived larvicidal agents. The use of leaf powder in solvents for the treatment reduces the cost of extraction and time for screening. This study may also contribute to assess the possibility of using large biomass of weeds available in the wastelands of northern India as potential insecticides in spite of using other medicinal or cultivable plants which are facing extinction or severe genetic loss. For improving the potency and stability of the products, in-depth investigation on the solvent extract and active compound of these plant species are needed for further elucidation.

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