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Research Article

Laboratory Evaluation of Local Plant Extracts and an Organophosphate Insecticide against Seabuckthorn Psyllid

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Abstract

Botanical extracts namely aqueous leaves extracts of *Artemisia brevifolia* (7.5%) and *Eupatorium adenophorum* (7.5%), aqueous drupes extract of *Melia azedarach* (7.5%); and malathion 50EC (0.025%) were evaluated for their efficacy against psyllid, *Cacopsylla* sp. (Hemiptera: Psyllidae) infesting seabuckthorn (*Hippophae* sp.) in Lahaul valley of Himachal Pradesh. Malathion 50EC (0.025%) caused up to 97.33 and 100 per cent psyllid mortality after 24 and 48h of its application, respectively. The psyllid mortality caused by leaves extracts of *A. brevifolia* (7.5%), *E. adenophorum* (7.5%) and drupes extract of *M. azedarach* (7.5%) was 60.27, 57.87, 60.27 and 75.73, 81.33, 81.60 per cent at 24 and 48h after application, respectively during 2011. The corresponding psyllid mortality during 2012 was 57.07, 57.33, 54.67 per cent at 24h and 80.80, 81.33, 73.07 per cent at 48h after application, respectively.

Keywords: Botanical extracts, *Cacopsylla*, efficacy, *Hippophae*, Himachal Pradesh

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Introduction

Psyllids or jumping plant lice are small phloem feeding insects having piercing and sucking mouth parts. The psyllids have been reported to vector phytoplasma diseases in horticultural plants. For instance, *Cacopsylla pyri* L., *C. pyricola* (Foerster) and *C. pyrisuga* (Foerster) are considered as important vectors of pear decline caused by phytoplasma [1-3] and *C. picta* (Foerster) as a vector of apple proliferation phytoplasma [4-6]. A psyllid, *Cacopsylla* sp. (Hemiptera: Psyllidae) infests seabuckthorn (*Hippophae rhamnoides* L.) growing naturally in high altitude regions of north west Himalayas [7]. While feeding, the nymphs and adults of this psyllid insert their mandibular and maxillary stylets into the young succulent leaves, inject saliva and then absorb the liquid food. Excess water with dissolved sugars is subsequently excreted as tiny white droplets of honeydew on to the leaves and stems. Copious honeydew secretions by colonies of this psyllid lead to sooty fungal growth. Consequently, the affected leaves and stems get blackened. The uncontrolled infestations often lead to poor plant vigour and reduced berry production.

Seabuckthorn is a very useful plant whose bark, berries, leaves and seeds possess many compounds like amino-acids, carbohydrates, carotenes, flavonoids, minerals, vitamins and volatile oils that are of paramount importance in pharmaceutical, nutraceutical and cosmetic industries [8-12]. The antioxidant properties of the flavonols of seabuckthorn berries such as isorhamnetin, kaempferol, myricetin, quercetin, rutin [13], tocopherols, tocotrienols [14, 15] and carotenoids [16, 17] have been experimentally established [18]. Also, seabuckthorn seeds are rich in carotenoids, fatty acids, flavonoids, kaempferol, organic acids, phytosterols, phenolic compounds, proanthocyanidins, sugar, triacylglycerol, vitamin C and vitamin E [19-21]. Various other extracts of this plant also exhibit marked antioxidant activity [22-27]. The nitrogen fixing ability coupled with wealth of nutrients contained in various parts of seabuckthorn make its cultivation potentially useful in improving the nutritional status of poor degraded soils and economic status of tribal farmers [28].

However, despite immense industrial utility, this plant somehow remained grossly unexploited in its natural habitat in north west Himalayan region. Even berries from the self perpetuating seabuckthorn stands growing on approximately 12000ha in these high altitude areas [29] are virtually wasted. Therefore, in the beginning vast natural seabuckthorn stands can be transformed into managed orchards. Afterwards, its scientific cultivation can be vigorously promoted. However, one of the major stumbling blocks in this endeavor is the psyllid infestation noted in natural seabuckthorn stands. Therefore, laboratory evaluation of some freshly prepared botanical extracts and an organophophate insecticide against the field collected nymphs was done to assess their potential lethal effects on nymphal population.

Materials and Methods

Tested botanical extracts/ insecticide and their doses

The botanical extracts evaluated against this sap sucking psyllid in the present studies included aqueous leaves extracts of *Artemisia brevifolia* (7.5%) and *Eupatorium adenophorum* (7.5%) and aqueous drupes extract of *Melia azedarach* (7.5%). The solitary insecticide tested was malathion 50EC (0.025%).

Procurement of botanicals (leaves and drupes) and preparation of extracts

The leaves of *A. brevifolia* were harvested from plants growing abundantly in the vicinity of Highland Agricultural Research and Extension Centre, Kukumseri, Lahaul and Spiti of Himachal Pradesh Agricultural University, Palampur, India during September and were shade dried. These dried leaves were powdered and stored in a paper bag at room temperature during winter months until their use in June/July next year. Whereas, the powdered leaves of *E. adenophorum* were procured from the Department of Organic Agriculture, Himachal Pradesh Agricultural University, Palampur. The powder of *E. adenophorum* leaves had also been prepared from the shade dried green leaves and stored in a transparent plastic container. On the other hand, the drupes of *M. azedarach* were collected from Una district of Himachal Pradesh. These drupes were directly harvested from plants in September/October and dried under shade. The dried drupes were then stored in gunny bags and brought to the laboratory in March/April. Dried powders of *A. brevifolia*, *E. adenophorum* leaves and broken drupes of *M. azedarach* each weighing 75g were soaked in separate glass containers containing one liter water for 24h to get 7.5 per cent solutions. After soaking, the contents were stirred several times with glass rod during the day and left undisturbed overnight. The next day, after soaking for the required duration, the contents were stirred one final time and sieved to get clear spray able extracts. For this, the coarse solid contents were first removed by simple plastic sieve. Subsequently, the extracts containing comparatively finer particles were filtered through single and multiple layers of muslin cloth to eventually get clear solutions.

Details of experimentation

The experiment was conducted in the Plant Protection Laboratory of Highland Agricultural Research and Extension Centre, Kukumseri (32°44'55" N latitude and 76°41'23" E longitude; 2772m amsl), Lahaul and Spiti of Himachal Pradesh Agricultural University, Palampur, India during summers of 2011 and 2012. The experiment was laid out in completely randomized design (CRD). Transparent plastic containers (10cm in diameter and 7cm deep) with tight fitting caps were used in the studies. The caps were manually perforated to prevent the psyllids from exiting the containers and in maintaining proper aeration during experimentation. A 'Whatman' filter paper of relevant size was placed at the bottom of each container. Subsequently, a succulent freshly incised seabuckthorn twig with intact green leaves was placed in the container. The cut ends of the twig were wrapped in water soaked cotton swabs to maintain its succulence. Twenty five field collected psyllids of apparently similar size were then released on it. Five replications per treatment were maintained along with unsprayed check (spray of tap water only) and each replication comprised of three containers. The desired aqueous extracts of leaves/drupes or the insecticide solution were thoroughly sprayed on to the released psyllids and perforated caps were screwed on the containers.

Data recording and statistical analysis

The data were recorded on numbers of alive psyllids 24 and 48h after spraying. The psyllids showing some movement were considered alive; whereas, others exhibiting no apparent signs of movement were considered dead. The numbers of dead psyllids were then worked out as under:

$$Pd = Pt - Pa$$

Where, *Pd*- number of dead psyllids; *Pt*- total number of psyllids released on to the twig in each plastic container; *Pa*- number of psyllids alive at n^{th} hour after treatment application (n = 24, 48). The replication wise numbers of dead psyllids were aggregated and averaged to find mean mortality after specified intervals. Similarly, replication wise per cent psyllid mortality was also worked out as under:

$$Pm = \{Pd / Pt\} * 100$$

Where, *Pm*- psyllid mortality (%); *Pd*- number of dead psyllids at n^{th} hour after treatment application (n = 24, 48); *Pt*- total number of psyllids released on to the twig in each plastic container.

Subsequently, the data on mean psyllid mortality (number of dead psyllids after 24 and 48h) were analyzed after square root transformation; whereas data on per cent psyllid mortality were analyzed after arc sine transformation using CPCS1 software [30].

Results and Discussion

Effect of botanical extracts and malathion 50EC application on psyllid mortality

At 24h after application

The results of the present studies revealed that malathion 50EC (0.025%) was more effective than botanical extracts against seabuckthorn psyllids in both the years. Malathion 50EC (0.025%) caused 97.33 and 94.67 per cent mortality of the psyllids at 24h after its application in 2011 and 2012, respectively. The corresponding numbers of dead psyllids were 73 and 71 out of 75 released per replication. Whereas, efficacy results of tested botanical extracts showed that all the three were statistically non-significant with each other in causing psyllid mortality at 24h in 2011. The psyllid mortality caused by applied botanical extracts at this time varied from 57.87-60.27 per cent during this year. The highest mortality (60.27%) in this year was recorded in psyllids that received sprays of *A. brevifolia* leaves extract (7.5%) as well as *M. azedarach* drupes extract (7.5%). Correspondingly, the mean count of dead psyllids after this time was 45.20 out of 75 released per replication. The mean number of dead psyllids recorded in *E. adenophorum* leaves extract (7.5%) was 43.40 out of 75 released per replication (Table 1). In the year 2012, leaves extracts of *A. brevifolia* (7.5%), *E. adenophorum* (7.5%) and drupes extract of *M. azedarach* (7.5%) caused 57.07, 57.33 and 54.67 per cent psyllid mortality, respectively. The corresponding mean count of dead psyllids was 42.80, 43 and 41 out of 75 released per replication. Meanwhile, the mortality of psyllids that were sprayed with water only was 2.40 and 3.20 per cent in 2011 and 2012, respectively. This corresponded to 1.80 and 2.40 mean number of dead psyllids out of 75 released in each container observed during this time in two years, respectively (Table 2).

At 48h after application

At this time, malathion 50EC (0.025%) sprayed containers recorded cent per cent mortality in both the years with no psyllid surviving the insecticide spray. Statistically, all the three botanical extracts differed significantly in causing psyllid mortality during both the years. In 2011, *M. azedarach* drupes extract (7.5%) and *E. adenophorum* leaves extract (7.5%) proved statistically non-significant but better than *A. brevifolia* leaves extract (7.5%) in causing psyllid mortality. The psyllids mortality recorded in respective botanical extracts was 81.60, 81.33 and 75.73 per cent during this year which corresponded to 61.20, 61 and 56.80 mean number of dead psyllids out of 75 released per replication. The control containers during this time recorded only 4 per cent psyllid mortality in the same year which corresponded to 3 mean numbers of dead psyllids out of 75 released per replication (Table 1). However, during 2012, leaves extracts of *E. adenophorum* (7.5%) and *A. brevifolia* (7.5%) were statistically non-significant but better than *M. azedarach* drupes extract (7.5%) in causing psyllid mortality. The psyllid mortality of 81.33, 80.80 and 73.07 per cent was recorded in three botanical extracts, respectively. The corresponding mean count of dead psyllids was 61, 60.60 and 54.80 out of 75 released per replication. The mortality in the control containers during this time was 3.73 per cent which corresponded to 2.80 mean numbers of dead psyllids out of 75 released per replication (Table 2).

The published literature does contain numerous reports of seabuckthorn infestation by psyllid, *Cacopsylla hippophaes* Foerster from different parts of the world [31- 33] but with no apparent concomitant damage to the plant as such. However, some degree of plant damage ascribed to this psyllid has been reported from Russia, China and a few other seabuckthorn growing countries [34]. But, studies on its effective management are lacking which could probably be due to its non-pest status in these countries. Some important plants attacked and damaged by psyllids include citrus [35-38]) pear [39, 40]), eucalyptus [41], fig [42], tomato/ potato [43, 44] and literature is replete with studies on their management. Therefore, present results have been discussed in light of these earlier studies on the occurrence and management of psyllids on different horticultural, forest and forage crops [45, 46]) of commercial importance using synthetic insecticides and organic products.

Table 1 Effect of botanicals (aqueous extracts) and insecticide (malathion 50EC) on the mortality of seabuckthorn psyllid in 2011

Botanical extract/ insecticide (Dose)	**Mortality**			
	No. of dead psyllids[1,3]		**Per cent mortality[2,3]**	
	24h	**48h**	**24h**	**48h**
Artemisia brevifolia leaves extract (7.5%)	45.20 (6.79)[b]	56.80 (7.60)[c]	60.27 (50.92)[b]	75.73 (60.64)[c]
Eupatorium adenophorum leaves extract (7.5%)	43.40 (6.67)[b]	61.00 (7.87)[bc]	57.87 (49.52)[b]	81.33 (64.54)[bc]
Melia azedarach drupes extract (7.5%)	45.20 (6.79)[b]	61.20 (7.89)[b]	60.27 (50.93)[b]	81.60 (64.65)[b]
Malathion 50EC (0.025%)	73.00 (8.60)[a]	75.00 (8.72)[a]	97.33 (82.79)[a]	100.0 (89.96)[a]
Control (water spraying only)	1.80 (1.66)[c]	3.00 (1.99)[d]	2.40 (8.71)[c]	4.00 (11.40)[c]
CD at 5%	0.31	0.28	5.02	3.90

[1]Figures in the parentheses are square root transformed means; [2]Figures in the parentheses are arc sine transformed means; [3]Figures in the parentheses followed by the same alphabet are statistically at par with each other.

Table 2 Effect of botanicals (aqueous extracts) and insecticide (malathion 50EC) on the mortality of seabuckthorn psyllid in 2012

Botanical extract/ insecticide (Dose)	**Mortality**			
	No. of dead psyllids[1,3]		**Per cent mortality[2,3]**	
	24h	**48h**	**24h**	**48h**
Artemisia brevifolia leaves extract (7.5%)	42.80 (6.61)[b]	60.60 (7.85)[b]	57.07 (49.06)[b]	80.80 (64.04)[b]
Eupatorium adenophorum leaves extract (7.5%)	43.00 (6.63)[b]	61.0 (7.87)[b]	57.33 (49.23)[b]	81.33 (64.46)[b]
Melia azedarach drupes extract (7.5%)	41.00 (6.48)[c]	54.80 (7.47)[c]	54.67 (47.66)[b]	73.07 (58.73)[c]
Malathion 50EC (0.025%)	71.00 (8.49)[a]	75.00 (8.72)[a]	94.67 (76.76)[a]	100.0 (89.96)[a]
Control (water spraying only)	2.40 (1.83)[d]	2.80 (1.94)[d]	3.20 (10.12)[c]	3.73 (11.04)[d]
CD at 5%	0.32	0.20	3.40	2.46

[1]Figures in the parentheses are square root transformed means; [2]Figures in the parentheses are arc sine transformed means; [3]Figures in the parentheses followed by the same alphabet are statistically at par with each other

Synthetic insecticides and psyllid mortality

Studies conducted under Florida conditions showed that low-volume ground applications of malathion effectively controlled Asian citrus psyllid, *Diaphorina citri* Kuwayama [47]. Rao *et al.* (2013a) [48] also recorded significantly lesser population of *D. citri* following foliar application of malathion 50EC (0.06%) when compared to field release of various stages of a chrysopid predator, *Mallada desjardinsi* (Navas) (Neuroptera: Chrysopidae) at different doses (eggs @ 2, 4, 6/ shoot, early instar larvae @ 2, 4/ shoot, adults @ 1, 2 pairs/ shoot). Similarly, Sathe and Kamble (2015) [42] observed that malathion 50EC (0.03%) was effective in controlling fig psylla, *Homotoma indica* Mathur (Homotomidae:Hemiptera) infesting *Ficus carica*. Likewise, another organophosphate insecticide chlorpyriphos caused more than 90 per cent mortality of overwintered psyllid adults which was greater than that caused by diflubenzuron, fenoxycarb and abamectin [49]. Marcic *et al.* (2008) [50] observed that non-systemic insecticide spirodiclofen was most effective against the first generation of pear psylla (*Cacopsylla pyri* L.). Single spray of spirotetramat 100SC applied at a dose of 2.25 l/ha effectively reduced the population of *C. pyri* by 75.3-91.4 and 83.7-97.6 per cent after one and two weeks of its application, respectively [51]. Thus, these earlier studies proved that many insecticides including malathion 50EC were effective in controlling psyllids damaging different plants. As such, high psyllid mortality caused by organophosphate insecticide malathion 50EC, as observed in present studies, stood corroborated by these earlier investigations.

Organic products and psyllid mortality

Efficacy results of aqueous extracts of *A. brevifolia* and *E. adenophorum* leaves and broken drupes of *M. azedarach* tested in the present studies could not be corroborated as published literature lacked references on their efficacy against psyllids. Therefore, the efficacy of these aqueous extracts has been discussed with respect to other organic materials evaluated against various psyllids which could be used in integrated management of this psyllid too. For instance, Qureshi *et al*. (2015) [52] tested some organic insecticides to control *D. citri* and found that danitol 2.4EC (16 oz/acre), pure spray green (2.5% v/v) and microthiol (15 lb/acre) significantly reduced the nymphal population of *D. citri*. The greatest residual control of nymphs was seen on trees treated with microthiol (15 lb/acre) and danitol 2.4 EC (16 oz/acre). Similarly, the fewest *D. citri* adults were also noticed on trees treated with danitol 2.4 EC (16 oz/acre) though these were not statistically different than diatomaceous earth (50 and 100 lb/100 gal). Qureshi and Stansly (2016) [53] evaluated the impact of some organic insecticides applied alone or with horticultural mineral oil and insecticidal soap on populations of *D. citri* and beneficial insects in bearing citrus trees during dormant and growing seasons in south west Florida.

These authors reported that population of *D. citri* was significantly reduced by application of pyganic alone or with 435 oil, M-pede and danitol. Pyganic with M-pede performed better than with 435 oil and both performed better than pyganic alone. Likewise, Rao *et al.* (2013b) [54] reported that *D. citri* population was significantly high in plants fertilized with inorganic fertilizer (24.0-36.36/5cm twig) than those treated with organic manure (15.91-33.34/5cm twig). Jorgensen *et al*. (2013) [55] tested several bio-rational insecticides (Organic JMS Stylet-Oil®, Excel Oil®, Eco-Oil®, Neem 600 WP and Sap Sucker Plus) in laboratory bio-assays to assess their effects on tomato/ potato psyllid mortality. These investigators observed that single spray of various bio-rational insecticides did not produce substantial variation in the number of psyllid eggs remaining ($P>0.28$ for all effects) at 13 days after release (DAR). However, number of nymphs on each plant varied strongly with tested bio-rational insecticide ($P=0.003$ for the main effect) and nymph numbers were lowest for Organic JMS Stylet Oil (mean=2.0/cage; $P=0.006$). After two sprays at 23 DAR, Organic JMS Stylet Oil® and Excel Oil® ($P<0.001$; $P=0.035$) significantly reduced the total nymphs compared with water spray only.

Mann *et al.* (2011) [56] observed that volatiles from crushed garlic chive leaves, garlic chive essential oil, garlic chive plants, wild onion plants and crushed wild onion leaves all repelled *D. citri* adults when compared with clean air. A blend of dimethyl trisulfide and dimethyl disulfide, the primary sulfur volatiles, in 1:1 ratio showed an additive effect on inhibition of *D. citri* response to citrus volatiles. The plant volatiles from *Allium* sp. did not affect the behaviour of the *D. citri* ecto-parasitoid *Tamarixia radiata* (Waterston). These authors concluded that *Allium* sp. or the tri- and di-sulphides could be integrated into management programmes for *D. citri* without affecting natural enemies [56]. Tian *et al.* (2015) [57] demonstrated that clove essential oil and its constituents have potential as a source of natural insecticides.

In a laboratory bioassay, these authors found that clove essential oil, commercial eugenol (99%) and β-caryophyllene (98%) exhibited strong contact toxicity against the summer form adults of *Cacopsylla chinensis* (Yang and Li) with LD50 values of 0.730, 0.673 and 0.708 µg/adult, and against the nymphs with LD50 values of 1.795, 1.668 and 1.770 µg/nymph, respectively. Similarly, in field trials clove essential oil, commercial eugenol (99%) and β-caryophyllene (98%) caused 73.01 (4.80 mg/ml), 66.18 (2.40 mg/ml) and 46.56 (1.20 mg/ml) per cent reductions in population of *C. chinensis*, respectively [57]. In a screening program for insecticidal activity of the essential oils/ extracts derived from some Chinese medicinal herbs and spices, garlic (*Allium sativum* L.) essential oil was found to possess strong insecticidal activity against overwintering adults of *C. chinensis*. The essential oil of *A. sativum* possessed contact toxicity against overwintering *C. chinensis* with an LC50 value of 1.42 µg/adult. The two main constituent compounds, diallyl trisulfide and diallyl disulfide exhibited strong acute toxicity against the overwintering *C. chinensis* with LC50 values of 0.64 and 11.04 µg/adult, respectively [58]. Thus, all these past studies suggested that psyllids were susceptible to a wide array of organic materials and plant extracts. Hence, their efficacy indirectly corroborated the effectiveness of aqueous leaves extracts of *A. brevifolia* (7.5%), *E. adenophorum* (7.5%) and aqueous drupes extract of *M. azedarach* (7.5%) observed in present studies.

Conclusion

Several synthetic insecticides, organic materials and botanical extracts have been found effective against many psyllids belonging to diverse genra and species including *Cacopsylla* sp. As such, these synthetic insecticides and other organic products might also prove to be lethal to psyllid species infesting seabuckthorn. Therefore, these could probably be used in compatible manner to control psyllid on seabuckthorn in addition to aqueous leaves extracts of *A. brevifolia*, *E. adenophorum*, aqueous drupes extract of *M. azedarach* and malathion 50EC evaluated in present studies.

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