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Insect growth regulatory activities from oils of camphor and clove against *Spilosoma obliqua*

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Abstract: The present study was conducted to evaluate the insecticidal and growth regulatory activities of clove oil, *Syzygium aromaticum* and camphor oil, *Cinammomum camphora* against Bihar hairy caterpillar, *Spilosoma obliqua*. The fourth instar larvae were subjected to topical application by microapplicator syringe with doses of 2.5, 2.0, 1.5, 1.0 and 0.5 µl/larva. Camphor oil was most effective at 2.5, 2.0 and 1.5µl dose causing death of larvae ranging from 76-90%. It showed a strong growth regulatory activity with no adult emergence at the highest concentration. Clove oil was effective in reducing adult moth population to 27% at highest dose of 2.5 µl. Only this dose could cause larval mortality of about 60%. The study shows potential of these oils to be utilized as insecticides or antibiosis mechanism to suppress the population of *S. obliqua*.

Keywords: Botanicals, Camphor oil, Clove oil, Growth Regulatory Activity, *Spilosoma obliqua*

INTRODUCTION

The Bihar hairy caterpillar, *Spilosoma obliqua* is highly polyphagous pest feeding on almost all green vegetation. The major hosts include groundnut, sunflower, cashew, castor, cucurbits, mulberry, pigeon pea, other pulses, millets, etc.. The larvae are gregarious feeding on leaves and in severe infestation the whole crop is defoliated. For long time, the chemical pesticides have been the sole control measures which have given rise to many well-known and serious problems, including genetic resistance of pest species, toxic residues in stored products, increasing costs of application, hazards from handling, environmental pollution (Rembold, 1994). Bihar hairy caterpillar has shown certain level of behavioural resistance to different classes of insecticides and it is due to this reason the pest is difficult to control (Mondal *et al.,* 2013). In this context, botanical pesticides such as plant essential oils exert a wide range of behavioural and physiological effects on colonization, development, growth, survival and multiplication of insects. Essential oils are effective as contact and fumigant insecticides to a majority of insect pests giving quick knock down action above 1% concentration. Mostly components of essential oils are lipophilic in nature, which acts like oviposition deterrents, feeding deterrents, repellents and toxins to a broad range of insect pests. Essential oils offer an alternative source of insect control agents because they contain a range of bioactive chemicals, most of which are selective and have little or no harmful effect on the environment and the non-target organisms (Arshad *et al.*, 2014). This study was of great interest to investigate some alternative natural methods exploiting essential oils as substitutes for insecticides in the control of *Spilosoma obliqua*.

MATERIALS AND METHODS

The Dried flower buds of *Syzygium aromaticum* (clove) and leaves of *Cinnamomum camphora* (camphor) were obtained from Medicinal Plants Research and Development Centre (MRDC), G.B.Pant University of Agriculture & Technology, Pantnagar Uttarakhand. The oil from flower buds and leaves were extracted by hydrodistillation method (Ray *et al*., 2008).The distilled oil was separated from water by funnel and stored in refrigerator for further use. Adults of test insects *S.obliqua* were collected from Norman E. Borlaug Crop Research Centre of GBPUAT, Pantnagar. Insects were transferred separately to glass jars having an inner lining of white paper. Cotton soaked in 10 % sucrose solution kept in a small petri plate was put in the jar for adult feeding. The eggs so obtained were kept in plastic boxes and the neonate larvae were transferred to plastic tubs containing fresh and soft leaves of castor with the help of fine brush. Fresh food was supplied daily. Five doses of each of the oils 2.5, 2.0, 1.5, 1.0 and 0.5µl were topically applied to the thoracic region of cold immobilized individual fourth instar larvae by microapplicator syringe (Prajapati *et al*., 1998, Pavela, 2005., Tandon *et al*., 2008). Three replications were maintained. Control was untreated. The treated larvae were transferred to separate plastic boxes containing untreated castor leaves as food. The data on mortality was recorded at days after exposure (DAE). Moribund larvae were counted as dead. The other observations were recorded on the following parameters:- larval weight (g), larval period (d), pupal period (d), pupal weight (g), adult emergence (%) and terminal larval mortality (%). The growth and development parameters were analysed using one way ANOVA. Significant differences between treatments were determined using Duncan multiple range test (P≤0.05).

RESULTS AND DISCUSSION

Camphor oil *C. camphora* was most effective in its insect growth regulatory activity against Bihar hairy caterpillar. Mean larval weight (Table 1) was significantly reduced at 2.5, 2.0 and 1.5µl/larva dose with values being 0.07, 0.11 and 0.049g as compared to untreated control (0.289g). Larval mortality was highest (66.66%) at 2.5µl dose. Due to the application of oil at thoracic region of the larvae the larval hairs were lost, the skin turned black , larvae failed to moult and finally died at doses above 1.5µl . Larval period and pupal period were at par to control. The terminal larval mortality (90%) which indicated death of larvae before reaching pupation was highest at 2.5µl (Table 2). The oil reduced pupation per cent at 2.5, 2.0 and 1.5µl/ larva , to 10.23, 23.33 and 23.33% respectively. The same response was reported during adult emergence .None of the moths could emerge at 2.5µl dose. Control recorded 96.66 % adults while only 13.33% moths emerged at 2.0µl dose. The toxicity in camphor

Table 1. Effect of camphor oil on growth of fourth instar larvae of *Spilosoma oblique.*

Plant oil	Conc. (µl)	Mean weight /larva 2 DAE(g)	Mean weight gain/ larva 2 DAE (g)	Larval mortality (%)
Cinnamomum camphora	2.5	$0.388{\pm}0.10^{a}$	$0.073{\pm}0.10^{a}$	$66.66{\pm}23.09^{c}$
	2.0	$0.499{\pm}0.02^{ab}$	$0.119{\pm}0.02^{ab}$	$33.33{\pm}20.81^{b}$
	1.5	$0.429{\pm}0.05^{a}$	$0.049{\pm}0.05^{a}$	$26.66{\pm}15.27^{ab}$
	1.0	$0.569{\pm}0.08^{bc}$	$0.189{\pm}0.08^{bc}$	$0.00{\pm}0.00^{a}$
	0.5	$0.653{\pm}0.05^{c}$	$0.273{\pm}0.05^{d}$	$0.00{\pm}0.00^{a}$
Control (Untreated)	-	$0.669{\pm}0.01^{c}$	$0.289{\pm}0.01^{d}$	$0.00{\pm}0.00^{a}$

Within column, means ± SD followed by the same letter do not differ significantly using DMRT, P≤0.05, DAE= Days after exposure

Table 2. Effect of camphor oil on development of fourth instar larvae of *Spilosoma obliqua*

Plant oil	Conc. (µl)	Larval period (d)	Terminal larval mortality (%)	Pupal period (d)	Mean pupal weight (g)	Pupation (%)	Adult emergence (%)
Cinnamomum camphora	2.5	$21.00{\pm}7.47^{ab}$	$90.00{\pm}10.00^{c}$	$10.66{\pm}0.57^{ab}$	$0.257{\pm}0.23^{a}$	$10.00{\pm}10.00^{a}$	$0.00{\pm}0.00^{a}$
	2.0	$21.17{\pm}9.25^{b}$	$76.66{\pm}15.27^{c}$	$10.77{\pm}0.38^{b}$	$0.456{\pm}0.06^{a}$	$23.33{\pm}15.27^{a}$	$13.33{\pm}11.54^{a}$
	1.5	$21.19{\pm}10.07^{b}$	$76.66{\pm}11.54^{c}$	$11.00{\pm}0.00^{b}$	$0.207{\pm}0.19^{a}$	$23.33{\pm}11.54^{a}$	$23.33{\pm}32.14^{ab}$
	1.0	$20.73{\pm}10.40^{a}$	$26.66{\pm}15.27^{b}$	$11.00{\pm}0.00^{a}$	$0.390{\pm}0.05^{b}$	$73.33{\pm}15.27^{b}$	$53.33{\pm}23.09^{bc}$
	0.5	$20.93{\pm}10.59^{ab}$	$10.00{\pm}10.00^{ab}$	$10.66{\pm}0.577^{ab}$	$0.390{\pm}0.035^{a}$	$90.00{\pm}10.00^{bc}$	$60.00{\pm}20.00^{c}$
Control (Untreated)	-	$21.00{\pm}10.36^{ab}$	$0.00{\pm}0.00^{a}$	$10.00{\pm}0.00^{a}$	$0.386{\pm}0.033^{a}$	$100{\pm}0.00^{bc}$	$96.66{\pm}5.77^{d}$

Within column, means ± SD followed by the same letter do not differ significantly using DMRT, P≤0.05.

Table3. Effect of clove oil on growth of fourth instar larvae of *Spilosoma obliqua*

Plant oil	Conc. (µl)	Mean weight /larva 2 DAE (g)	Mean weight (g) gain/ larva 2 DAE	Larval mortality (%)
Syzygium aromaticum	2.5	$0.608{\pm}0.10^{a}$	$0.340{\pm}0.10^{a}$	$60.00{\pm}10^{c}$
	2.0	$0.640{\pm}0.03^{a}$	$0.357{\pm}0.03^{a}$	$23.33{\pm}5.77^{b}$
	1.5	$0.625{\pm}0.03^{a}$	$0.372{\pm}0.03^{a}$	$16.66{\pm}15.27^{b}$
	1.0	$0.709{\pm}0.01^{ab}$	$0.441{\pm}0.01^{ab}$	$0.00{\pm}0.00^{a}$
	0.5	$0.711{\pm}0.001^{ab}$	$0.443{\pm}0.001^{ab}$	$0.00{\pm}0.00^{a}$
Control (Untreated)	-	$0.745{\pm}0.05^{b}$	$0.477{\pm}0.05^{b}$	$0.00{\pm}0.00^{a}$

Within column, means ± SD followed by the same letter do not differ significantly using DMRT, P≤0.05.., DAE= Days after exposure

Table 4. Effect of clove oil on development of fourth instar larvae of *Spilosoma oblique.*

Plant oil	Conc. (µl)	Larval period (d)	Terminal larval mortality (%)	Pupal period (d)	Mean pupal weight (g)	Pupation (%)	Adult emergence [On the basis of pupation] (%)
Syzygium aromaticum	2.5	20.00±0.00[a]	70.00±0.00[c]	11.55±0.50[a]	0.367±0.02[a]	26.66±15.27[a]	26.66±15.27[a]
	2.0	20.47±0.13[a]	46.66±20.81[b]	11.52±0.50[a]	0.393±0.12[a]	53.33±20.81[b]	36.66±30.55[ab]
	1.5	20.58±0.52[a]	36.66±5.77[b]	12.80±0.72[b]	0.476±0.01[a]	63.33±5.77[b]	50.00±26.45[ab]
	1.0	20.75±0.05[a]	10.00±10.00[a]	12.91±0.97[b]	0.445±0.02[a]	90.00±10.00[c]	66.66±15.27[bc]
	0.5	20.63±0.55[a]	10.00±10.00[a]	12.77±0.58[b]	0.519±0.16[a]	90.00±10.00[c]	86.66±5.77[c]
Control (Untreated)	-	20.13±0.75[a]	0.00±0.00[a]	12.63±0.25[ab]	0.440±0.01[a]	100.00±0.00[c]	100.00±0.00[c]

Within column, means ± SD followed by the same letter do not differ significantly using DMRT, P≤0.05.

oil may be due to the presence of fenchone (34.82%), camphene (23.77%), α- thujene (17.45%), L-limolene (7.54%) and cis-p-methane (5.81%) as reported by Srivastava et al (2008). Cinnamaldehyde, eugenol, cinnamyl acetate and essential oils from different *Cinnamomum* species are effective mosquito larvicides (Huang *et al*., 1998, Cheng *et al*., 2004). The toxicity of clove *S. aromaticum* oil on growth and development parameters is depicted in tables 3 and 4, the lower doses were at par to control in terms of reduction in weight gain. Per cent reduction in weight gain was highest (28.73%) at 2.5µl and lowest (7.13%) at 0.5 µl in comparison to control (mean larval weight gain 0.47g).The highest dose viz. 2.5µl was most effective causing more than average larval (60%) and terminal larval (70%) mortality. There was no significant effect on larval and pupal durations. Low pupation per cent was noticed at 2.5 (26.66%), 2.0 (53.33%) and 1.5µl (63.33%). The oil reduced adult moth population by 26.66, 36.66 and 50% at 2.5, 2.0 and 1.5µl doses respectively. Clove bud oil has biological activities, such as antibacterial, antifungal, insecticidal and antioxidant activities and are used traditionally as flavoring agents and antimicrobial material in food (Lee & Shibamoto, 2001., Huang & Ho, 2002). Several compounds from *S.aromaticum* (namely5,7-dihydroxy-2-methylchromone-8-C-D-glucopyranoside, biflorin, kaempferol, rhamnocitrin, myricetin, gallic acid, ellagic acid and oleanolic acid) have been found to possess growth inhibitory activity against oral pathogens (Cai & Wu, 1996). In our study *C. camphora* and *S. aromaticum* oils were effective in controlling insect development at doses above 1.5µl. These oils could be effectively used in pest management programmes as potential insecticides, though we could not find enough research findings on the toxicity of these oils to *S. obliqua*. Workers have reported toxicity of these essential oils against other moths, for example *C.zelanicum* has been reported to be toxic to the larvae of Indian meal moth *Plodia interpunctella* (Rafiei *et al*, 2009a., Rafiei *et al*., 2009b). *C.camphora* oil has also been reported to be toxic to *Sitotroga cerealella* and *Ephestia kuehniella*. (Allahvaisi *et al*., 2011).*S. aromaticum* essential oil exhibited inhibition of F1 progeny from 61.08 to 91.52% against *Sitophilus oryzae* (Sharma & Meshram, 2006). Clove oil has also been reported to show contact and fumigant toxicities against adults of *Musca domestica* (Pavela, 2008), ovicidal effect against *Tribolium castaneum* (Mandal & Khalequzzaman, 2009), repellent activity against *Blattella germanica*, *Periplaneta americana* and *P. fuliginosa* (Yoon *et al*, 2009), larvicidal against both pyrethroid-susceptible and resistant *Ades aegypti* (Sutthanont *et al*., 2010), repellency to adults and larvae and ovicidal, larvicidal and adulticidal against *Tribolium castaneum* (Ajayi & Olonisakin, 2011), toxicity against the workers of the *Odontotermes obesus* termite (Gupta *et al*., 2011) *S.aromaticun* oil have been found to be repellent to fifth instar larvae of *Sitotraga cerealella* and *Ephestia kuehniella* and strong feeding deterrent activity have been reported against *Trichoplusia ni* (Akhtar *et al.*, 2012).The study shows the oils of *C.camphora and S.aromaticum*, if used at a higher concentration could serve as effective insecticides and insect growth regulators impairing normal development of agriculturally important insect defoliators.

Conclusion

It is clearly evident that oils from camphor and clove were highly potent in their insecticidal activity against Bihar hairy caterpillar. The oils showed IGR activity above 1.5µl dose/larva. Camphor oil was more effective at 2.0 & 2.5 µl. causing about 76 and 90% terminal larval mortality respectively with only 10% of conversion of larvae into pupae at the highest dose of 2.5µl/larva. No adults were formed at this dose of camphor oil. As compared to camphor oil, clove oil was less toxic causing about 70% terminal larval mortality at the highest dose of 2.5µl/larva allowing 27% of adult emergence. Utilization of camphor and clove oil in pest management programmes will reduce reliance on synthetic pesticides as well as hamper build up of resistance in insect populations. The use of oils is also compatible with integrated pest management tactics for sustainable agriculture with minimum risk to the ecosystem.

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