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Amit Paschapur
ICAR-Vivekananda Parvatiya Krishi Anusandhana Sansthan, Almora, Uttarakhand, India

ARNS Subbanna
ICAR-Vivekananda Parvatiya Krishi Anusandhana Sansthan, Almora, Uttarakhand, India

Ashish Kumar Singh
ICAR-Vivekananda Parvatiya Krishi Anusandhana Sansthan, Almora, Uttarakhand, India

Jeevan B
ICAR-Vivekananda Parvatiya Krishi Anusandhana Sansthan, Almora, Uttarakhand, India

J Stanley
ICAR-Vivekananda Parvatiya Krishi Anusandhana Sansthan, Almora, Uttarakhand, India

KK Mishra
ICAR-Vivekananda Parvatiya Krishi Anusandhana Sansthan, Almora, Uttarakhand, India

Corresponding Author:
Amit Paschapur
ICAR-Vivekananda Parvatiya Krishi Anusandhana Sansthan, Almora, Uttarakhand, India

Baseline susceptibility of major sucking pests of hill crops in north western Himalayan region

Amit Paschapur, ARNS Subbanna, Ashish Kumar Singh, Jeevan B, J Stanley and KK Mishra

Abstract
Greenhouse whitefly (*Trialeurodes vaporariorum*), green peach aphid (*Myzus persicae*) and cabbage aphid (*Brevicoryne brassicae*) are the three major sucking pests that infect hill crops in North Western Himalayan region and cause severe yield losses. Chemical management tactics are the widely practiced methods for management of these pests in the locality. However, knowledge about the efficacy of different pesticides against these native populations is lacking. In view of this, seven insecticides against whitefly and six against aphids were tested for their bio-efficacy under laboratory conditions. The results showed that, field populations of greenhouse whitefly were highly susceptible to three insecticides; Thiomethaxam> Imidacloprid> Pymetrozine with LC_{50} values 12.30, 18.62 and 22.38 ppm respectively. Whereas, LC_{50} values of botanical insecticides NSKE and nimbicidine were very high; 524.81ppm and 4365.16ppm respectively, indicating their non-suitability against greenhouse whitefly management. The susceptibility for B. brassicae, a major pest of crucifers is in the following order; Thiomethaxam> Imidacloprid> Indoxacarb> Pymetrozine> Nimbicidine> NSKE and Thiomethaxam was the most toxic insecticide with LC_{50} and LC_{90} values as low as 0.024ppm and 0.25ppm respectively. Green peach aphid (M. persicae) was highly susceptible to Thiomathaxam and was followed by Pymetrozine, indoxacarb, Imidacloprid, Nimbicidine and NSKE respectively. The LC_{50} values of Thiomethaxam and Pymetrozine were at par with a recorded value of 2.54ppm and 2.57ppm, respectively. The three sucking pests under study are well known for their resistance development mechanisms against several groups of insecticides. But, the level of susceptibility that was recorded in our study indicates presence of susceptible population of these sucking pests in Indian Himalayan region that could help to maintain a refugee or buffer populations against development of insecticide resistant strains.

Keywords: Greenhouse whitefly, green peach aphid, cabbage aphid, susceptibility, lethal concentrations, north western Indian Himalayas

1. Introduction
Greenhouse whitefly (*Trialeurodes vaporariorum*), green peach aphid (*Myzus persicae*) and cabbage aphid (*Brevicoryne brassicae*) are the three most important sucking pests in hill agriculture (Kim *et al*., 1986; Hill, 1987) [1, 2], wherein, the former two being polyphagous pests infecting large number of crops in both open field and greenhouse conditions, while the latter being a stenophagous in habit, infecting cruciferous crops. The sucking pests cause damage in three ways *viz.*, reduction in vitality due to loss in cell sap; inhibition inphotosynthesisdue to sooty mould and transmission of lethal viral diseases (Johnson *et al*., 1992; Blackman and Eastop, 2000; Castillo *et al*., 2011; Cavalieri *et al*., 2014) [3, 4, 5, 6]. The well-evolved reproduction (parthenogenesis in aphids and arrhenotokous parthenogenetic reproduction in whiteflies) and survival strategies (pseudopupa/ prepupal stage in whiteflies) in these pests make them superior even under adverse climatic conditions.
Although, several management strategies are available to suppress the pests on crops; efficacy, availability and cost of operations are not comparable with chemical control measures. Besides these are the last resorts to suppress any type of pest populations in order to avoid economic damage. Therefore, integrated pest management systems designed to suppress sucking pests on various crops still include insecticides as an important component (Liu *et al*., 1993; Toscano *et al*., 1998) [7, 8]. However, indiscriminate use of insecticides leads to the development of resistance to various organophosphates, carbamates and synthetic pyrethroids (Elhag and Horn, 1983; Buitrago *et al*., 1994; Zheng and Gao, 1995; Denholm and Jespersen, 1998) [9, 10, 11, 12]. Intensive research has been carried out in recent years for evaluating insecticides with novel modes of action against sucking pests due to low pace of resistance

development to these insecticides. Although, resistance and resurgence in sucking pests is becoming widespread due to their indiscriminate use (Nauen and Denholm, 2005) [13]. In spite of number of publications re-establishing toxicity for a range of compounds that include imidacloprid and other novel insecticides; the lack of up-to-date resistance and susceptibility monitoring data for *T. vaporariorum*, *B. brassicae* and *M. persicae* is a subject of concern (Bi *et al*., 2002; Wang *et al*., 2003) [14, 15].

In hill agriculture, farmers either use botanicals and/or safer novel insecticide molecules, which can manage the insect pests at lower dosages and in turn have least residues in the soil, agriculture produce and environment. Taking this into consideration; seven insecticide molecules popularly used by farmers in the locality to manage the above three sucking pests were selected and their acute toxicities were calculated for field populations of aphids and whiteflies collected from ICAR-VPKAS, experimental farm, Hawalbagh (29.63^{0}N and 79.63^{0}E, 1250 amsl) representing North Western Himalayan region. The median lethal concentrations for all the insecticides were calculated and the most effective among them was recommended to farmers for managing respective insect pests.

2. Materials and Methods

A. Insects: Adults of greenhouse whitefly (*Trialeurodes vaporariorum*), green peach aphid (*Myzus persicae*) and cabbage aphid (*Brevicoryne brassicae*) infecting tomato, capsicum and cauliflower respectively, were collected from open fields or polyhouses at ICAR-VPKAS, experimental farm, Hawalbagh (29.63^{0}N and 79.63^{0}E, 1250 amsl). The field populations, after confirming the identity, were tested directly without rearing in the laboratory. Apterous adults of aphids and winged adults of whiteflies were used for the study.

B. Insecticides: Seven most commonly used insecticide formulations to manage sucking pests in Uttarakhand hills were selected for the study; Acetamiprid (Prime @ 20% SP; Crop chemicals), Imidacloprid (Sacdor @ 17.8%SL**;** Shivalik crop sciences), Thiomethaxam (Dxstar @ 25%WG; Nagarjun Agrichemicals Ltd), Indoxacarb (King carb @ 14.5%SC; Parijat), Pymetrozine (Simca @ 50%WG; Adama), NSKE (Vanguard 1500@ 1500ppm azadirachtin EC; Agriland) and Nimbicidine (Nimbicidine 0.03%EC @ 300ppm azadirachtin; Stanes). All the insecticides were purchased from local dealers. All these seven insecticides were tested against whiteflies and six insecticides except acetamiprid were tested against aphids.

C. Bioassay: Leaf dip bioassay was conducted for all the insecticides with seven different concentrations ranging from 0.05ppm to 15ppm for aphids and 0.1ppm to 100ppm for whiteflies for each insecticide and each treatment was replicated three times. A control treatment was set up with double distilled water as treatment at each time. Respective host leaf discs of approximately 7.5cm diameter were dipped in insecticide solution for 1 minute and air dried for 5-10 minutes at room temperature (25±2^{0}C). The treated leaves were placed in a Petri dish (9cm diameter) with adaxial side of the leaf facing upwards and with a moist filter paper beneath to maintain humidity. Ten numbers of insects were released with a camel brush on each leaf and care was taken to avoid any physical damage while transferring. The lid with minute perforations was closed and plates were incubated at 25±2^{0}C for 96 hours.

3. Data analysis: Mortality of the adults was recorded at every 24 hours of insecticide exposure and was corrected by Abbott's (1925) [16] formula. Insects showing no movements after gentle touch with the camel brush were considered dead. Thus obtained data was subjected to probit analysis (Finney, 1971) [17] using the software package PoloPlus (LeOra Software 2013)

4. Results

Susceptibility of *T. vaporariorum* to insecticides: Field populations of greenhouse whitefly were highly susceptible to three insecticides; Thiomethaxam> Imidacloprid> Pymetrozine. The LC_{50} values for botanical insecticides NSKE and nimbicidine were very high, 524.81ppm and 4365.16ppm respectively. Whereas, LC_{50} values for acetamiprid, the most commonly used insecticide in North Western Himalayan region for management of greenhouse whiteflies was 38.02ppm. Indoxacarb, an insecticide used by farmers to manage both American tomato pin worm (*Tuta absoluta*) and *T. vaporariorum* in polyhouses showed LC_{50} value of 52.48ppm. The details of the probit analysis along with LC_{50} and LC_{90} are mentioned in table 1.

Table 1: Toxicity of selected insecticides against field populations of *Trialeurodes vaporariorum* collected from ICAR-VPKAS, Experimental farm, Hawalbagh, Almora, Uttarakhand

Insecticide	Linear equation (Y= ax+b)	Slope±SE	χ^2	Df	LC_{50} (in ppm)	LC_{90} (in ppm)
Acetamiprid	Y=2.51x+1.02	2.51±0.77	0.76	6	38.02	123.03
Imidacloprid	Y =2.73x+1.52	2.73±0.51	0.90	6	18.62	54.92
Thiomethaxam	Y =2.85x+1.87	2.85±0.50	0.91	6	12.30	35.48
Indoxacarb	Y =2.36x+0.93	2.36±0.72	0.76	6	52.48	467.74
Pymetrozine	Y =2.43x+1.71	2.43±0.44	0.90	6	22.38	75.86
NSKE	Y =1.70x+0.38	1.70±0.74	0.62	6	524.81	2951.21
Nimbicidine	Y =1.35x+0.08	1.35±0.80	0.47	6	4365.16	38904.51

Susceptibility of *B. brassicae* to insecticides: *B. brassicae* a major aphid pest of crucifers in North western Himalayan region was highly susceptible to all the six tested insecticides including botanical insecticides, NSKE and nimbicidine. The baseline susceptibility decreased in the following order; Thiomethaxam>Imidacloprid>Indoxacarb>Pymetrozine> Nimbicidine> NSKE. Thiomethaxam was the most toxic insecticide with LC_{50} and LC_{90} values as low as 0.024ppm and 0.25ppm respectively. The details of the probit analysis along with LC_{50} and LC_{90} are mentioned in table 2.

Table 2: Toxicity of selected insecticides against field populations of *Brevicoryne brassicae* collected from ICAR-VPKAS, Experimental farm, Hawalbagh, Almora, Uttarakhand

Insecticide	Linear equation (Y= ax+b)	Slope±SE	χ^2	Df	LC_{50} (in ppm)	LC_{90} (in ppm)
Imidacloprid	Y=1.13x+5.12	1.13±0.08	0.96	6	0.78	10.47
Thiomethaxam	Y=1.26x+7.05	1.26±0.23	0.82	6	0.024	0.25
Indoxacarb	Y=1.43x+4.99	1.43±0.32	0.76	6	1.02	7.94
Pymetrozine	Y=1.29x+5.36	1.29±0.26	0.79	6	1.9	8.91
NSKE	Y=1.88x+4.03	1.00±0.37	0.81	6	3.31	15.49
Nimbicidine	Y=1.42x+5.44	1.42±0.22	0.87	6	2.04	13.89

Susceptibility of *M. persicae* to insecticides: *M. persicae* a major polyphagous aphid pest infecting more than 100 crops in hill region showed almost uniform susceptibility to all the six insecticides tested. Thiomathaxam being the most toxic insecticide followed by Pymetrozine, indoaxacarb, Imidacloprid, Nimbicidine and NSKE. The LC_{50} values for Thiomethxam and Pymetrozine were on par with each other (2.54ppm and 2.57ppm) respectively. The details of the probit analysis along with LC_{50} and LC_{90} are mentioned in table 3.

Table 3: Toxicity of selected insecticides against field populations of *Myzus persicae* collected from ICAR-VPKAS, Experimental farm, Hawalbagh, Almora, Uttarakhand

Insecticide	Linear equation	Slope±SE	χ^2	Df	LC_{50}	LC_{90}
Imidacloprid	y=2.69x+2.71	2.69±0.37	0.89	6	7.07	21.38
Thiomethaxam	Y=2.93x+3.81	2.93±0.36	0.91	6	2.54	6.92
Indoxacarb	y=2.37x+3.1	2.37±0.22	0.95	6	6.31	21.87
Pymetrozine	Y=2.95x+3.79	2.95±0.35	0.92	6	2.57	6.92
NSKE	y=2.46x+2.58	2.46±0.38	0.87	6	9.55	31.62
Nimbicidine	Y=2.28x+2.98	2.28±0.21	0.95	6	7.58	28.18

All in all the bioassay results against three major sucking pests of North Western Himalayan region showed that thiomethaxam (Dxstar @ 25%WG; Nagarjun Agrichemicals Ltd) was the most toxic among the seven insecticides tested against both whitefly and two aphid species. The botanical formulations NSKE (Vanguard 1500 @ 1500ppm azadirachtin EC; Agriland) was least toxic to aphid species and Nimbicidine (Nimbicidine 0.03%EC @ 300ppm azadirachtin; Stanes) was least toxic to greenhouse whitefly. All these mortalities were recorded at 24 hours after the treatment, which indicates that the aphid and whitefly populations were highly susceptible to the major insecticides available in the market and adapted by the farmers.

5. Discussion
The three sucking pests under study are well examined for their dynamic physiology of developing resistance to major conventional insecticides like organophosphates, carbamates and pyrethroids (Zou and Zheng, 1988; Omer *et al.*, 1992) [18] [19]. *Myzus persicae* is known for overproduction of insecticide-detoxifying carboxylesterases, resulting from structural amplification of genes encoding these enzymes (Foster *et al*, 2000) [20]. Ahmad and Aslam, (2005) [21] showed that *Brevicoryne brassicae* populations from Pakistan were resistant to major organophosphates and pyrethroids. Whereas, enhanced production of P450 CYP6CM1 in *T. vaporariorum* has shown resistant to all conventional insecticides along with few neonicotinoids (Imidacloprid) and even pymetrozine (Karatolos *et al*, 2010) [22] indicating the scenario parallel to *Bemisia tabaci*. Among the reports of insecticide resistance development, those populations collected from Indian Himalayas are known to be highly susceptible to novel insecticides (Sood *et al*, 2006) [23] and the present research findings are in agreement with this. However, botanicals were not of much importance in whitefly management due to higher LC_{50} values recorded for NSKE and Nimbicidine respectively. Whereas, the three neonicotinoid insecticides, Imidacloprid, acetamiprid and thiomethxam were highly toxic and are a welcome new class of chemistry for aphid and whitefly control. Indoxacarb a novel molecule recommended for coleopteran and lepidopteran pest management had significant toxicity to sucking pests (Homoptera) which shows its broad activity spectrum. Pymetrozine a specialised sucking pest control molecule proved to be a promising insecticide molecule and recorded lower LC_{50} values that are at par with neonicotinoids.

In conclusion, the insect pest populations of Indian Himalayas are least exposed to pesticide treatments and are susceptible to most of novel molecules. This status of susceptibility has to be maintained by discriminate and wise use of available insecticides to avoid development of resistant populations.

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