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Screening of wild *Ipomoea* genotypes for resistance against sweet potato weevil *Cylas formicarius* F. based on multiple choice bioassay and phytochemical constituents

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ABSTRACT: Screening of wild *Ipomoea* spp. and identification of new sources of resistance to the sweet potato weevil (*Cylas formicarius* Fabricius) with *I. palmata, I. mauritiana, I. obscura, I. triloba* were carried out. The leaves, vines and tubers of the different *Ipomoea* sp. were screened using multiple choice bioassay. The insect feeding holes on *I. mauritiana* leaves (1.67+1.528), vines (7.67±2.96) and tubers (12.67+2.309) was significantly less compared with other *Ipomoea* sp. Further, the two-choice bioassay was done, using *I. batatas* and *I. mauritiana* for comparison. Based on the morphological screening different phytochemical constituents was identified using GC-MS analysis of the methanolic extract of roots of selected *Ipomoea* spp. (*I. mauritiana, I. palmata* and *I. batatas*). The results indicated that the phytochemical constituent of *I. mauritiana viz*., undecane, quinic acid which is to have insecticidal activity. The major constituent of *I. batatas* comprises of melezitose (38.53%) and alpha-I-rhamnopyranose (21.26%). It can be concluded that the phytochemical constituents of *I. mauritiana* was responsible for the antibiosis.

KEYWORDS: Antibiosis, bioactive, insecticidal, bioassay, undecane

INTRODUCTION

The weevil *Cylas formicarius* F. belonging to Coleoptera, Brentidae, is a destructive pest of sweet potato and is widely spread throughout the tropical regions of the world, but the methods of control are the significant problem faced by growers in most countries producing sweet potato. Generally, weevils cause severe feeding destruction to sweet potato roots, vines, stems and leaves through their life cycle, beginning from the egg stage to adult stage. Weevil infested tubers are bitter due to the production of a terpene compound and the infested tubers are unfit for consumption or convert to livestock, resulting major economic losses (Uritaini *et al.,*1975; Palaniswami and Mohandas, 1993; Korada *et al*., 2010a; Kyereko *et al.,* 2019). Although *C. formicarius* prefers sweet potato,

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more than 30 species of *Ipomoea* and other genera have been recorded as its host plants (Sutherland, 1986; McConnell and Hossner, 1991). About 500-600 species were included in the genus *Ipomoea* sp. within the Family Convolvulaceae (Austin and Huáman, 1996). Studies have proved that the management sweet potato weevil (SPW) can be done by integrated pest management *viz.,* removal and destruction of hosts, cultural methods, biological control, botanicals, chemical pesticides, tolerant varieties and use of semiochemicals (Palaniswami *et al.,* 1992; Pillai *et al.,* 1993; Palaniswami and Chattopadhyay, 2006; Korada *et al.,* 2010a).

Earlier studies on the identification of the resistant sweet potato genotypes to the weevil indicated only relatively tolerant ones. Studies conducted at AVRDC and Penghu Island has reported screening of the population *I. trifida* x *I. batatas* hybrids with high yield and low weevil infestation (Talekar, 1987). An indigenous cultivar Selopia was identified moderately resistant to the weevil by screening based on crown damage grade index (DGI), percentage tuber damage, tuber DGI, adult emerged per kg infested tuber (Palaniswami and Mohandas 1992). Korada *et al*. (2010b) reported that among the sweet potato genotypes, *viz*., Goutam, Sourin, Gouri and CIP-6 evaluated for SPW resistance, CIP-6 was the most susceptible. Further in their electroantennogram studies identified the electrophysiological response of female antenna to the volatile extracts of aerial plant parts and roots was higher than the male antenna of the weevil. In olfactometer studies, the headspace volatiles of genotype CIP-6 attracted more number of female *C. formicarius* weevils than volatiles of Gouri, Goutam and Sourin. Variation in the preference of sweet potato genotypes to *C. formicarius* is attributed to differential emission of volatiles from the aerial parts and roots. Reddy *et al*. (2015) reported that the weevil, developed faster on *Ipomoea batatas* than on *I. triloba.*

Anyanga *et al*. (2013) found that hydroxycinnamic acid esters on the exterior and the root latex, decreases weevil's nourishment and oviposition providing resistance to SPW. Okada *et al*. (2019) identified genetic regions associated with weevil resistance in 90IDN-47 and PSL sweet potato genotypes by genome wide association studies (GWAS) in Japan. In their experiment on the degree of weevil damage to the genotypes, no single nucleotide polymorphisms (SNPs) were identified above the significance thresholds. However, one relatively high peak was found in the 90IDN-47 genotype, which showed resistance to weevils. On the other hand, one relatively high peak was also detected in the PSL genotype, which showed susceptibility to weevils. These results suggest that two regions could affect weevil resistance and may contain the gene(s) controlling weevil resistance. SPW can survive on average longer than four months on sweet potato as well as *I. triloba* (Reddy and Chi, 2015). Hence identification of host plant resistance source against weevil is one of the alternative strategies for the pest management. In the present study, genotypes from different species of *Ipomoea* were selected based on the reports (Reddy and Chi, 2015) on host preference by weevils and experiments were conducted to screen wild *Ipomoea* spp. for resistance against weevil based on the nature of feeding by sweet potato weevils and their phytochemical constituents.

MATERIALS AND METHODS

Multiple choice bioassay: Multiple choice bioassays (Vos and Jander, 2008) were carried out using leaves, vines and roots of plant species *viz Ipomoea batatas, I. mauritiana*, *I. palmata, I. obscura* and *I.triloba* Five plant samples were placed in large Petridish (180x30mm) and 20 weevils (@1male: 5females) were released in the centre of the Petridish. The sweet potato weevils were reared in Entomology laboratory and were used for bioassay. The position and readings of feeding holes by weevils (cumulative values) were recorded for three consecutive days. Experiment using leaves, vines and roots were conducted separately in three replications and each experiment was repeated thrice. Control samples (leaves, vines, roots) for each experiment were kept separately.

Two - choice bioassay: No-choice bioassays (Vos

and Jander 2008) were carried out using fresh leaves, vines and roots of sweet potato and *I. mauritiana.* The plant samples were placed in large Petridish (180x30mm) and 18 sweet potato weevils (1male: 5 females) were introduced to the centre of the Petridish. The sweet potato weevils were reared in Entomology laboratory and were used for bioassay. The position and readings of feeding holes by weevils (cumulative values) were recorded for three consecutive days. Experiment using leaves, vines and roots were conducted separately in three replications and each experiment was repeated thrice. Control samples (leaves, vines, roots) for each experiment were kept separately.

Data were subjected to analysis of variance using IBM SPSS version 21.The differences between the treatments was measured by tuckeys test at *P*_0.05, and the treatment means were compared using the least significant difference at 5 per cent. Data for no choice assay were subjected to t-test at *P*_0.05.

Gas chromatography-Mass spectrum analysis: Further for GC-MS analysis one tuberous wild *I. mauritiana*, one non-tuberous wild *I. palmata* and *I. batatas* were selected for the anlaysis. The required quantity of the whole plant tubers/roots was washed, air dried and weighed. It was transferred to a flask, treated with methanol of 500ml until the tubers was fully immersed, incubated overnight and filtered through a Whatmann No. 41 filter paper**.** Before filtering, the filter paper along was wetted with methanol. The filtrate is then concentrated to 5 ml using flash evaporator. The GC-MS analysis was done at Sophisticated Analytical Instruments Facility (SAIF), IIT, Chennai. GC-MS analysis of the methanol extract was performed using an Agilent-Technologies 8890 Network GC system equipped with an Agilent-Technologies 5977 mass selective detector (Agilent-Technologies, Little Falls, CA, USA). For MS detection, the electron ionization mode with ionization energy of 70 eV was used, with a mass range at m/z 50–600. An HP-5MS capillary column (30 m × 250 ìm, film thickness 0.25 ìm) was used for GC/MS. The column temperature was programmed from 180 to 300 °C at a rate of 5 °C/min with the lower and upper temperature being held for 3 and 5 min, respectively. GC was performed in the split mode. Helium was used as carrier gas at a flow rate of 1.2 ml/min. An injection 1 µl was used for each diluted extract. Essential compounds were identified by their retention times and mass fragmentation patterns using data of standards at NIST library

RESULTS AND DISCUSSION

Multiple choice bioassay: The weevil feeding holes on *I. mauritiana* was significantly less compared with *I. batatas*, *I. triloba. I. palmata* and *I. obscura*. The insect feeding holes on *I. mauritiana* leaves (1.67+1.52) was significantly low, when compared tpother *Ipomoea* species (Table 1). Similarly the same pattern was observed for the three consecutive days and mortality of insects was also observed. The insect feeding holes on *I. mauritiana* vines was less (7.67±2.96), compared to other *Ipomoea* species. The same pattern was observed for the three consecutive days given (Table 2). The insect feeding holes on *I. mauritiana* tubers was significantly low (12.67 ± 2.30), when compared to other *Ipomoea* species (Table 3).

Two-choice bio-assay: The weevil feeding holes on leaf, vines and tubers of *I. mauritiana* and *I. batatas* indicated great variation between them. *I. mauritiana* showed resistance to the weevil (Table 4).

Table 1. Leaf feeding (no. of holes) by the weevils on *Ipomoea* species in multiple choice bioassay

Species	1st Day	2nd Day	3rd Day
I. mauritiana	1.67± 1.52[a]	4.67±1.15[a]	7.33±0.57[a]
I. triloba	4.00± 1.00[a]	7.00± 1.00[a]	9.67± 0.57[a]
I. palmata	10.00 ± 2.64[b]	15.33± 2.30[b]	18.33± 2.88[b]
I. obscura	10.33± 2.08[b]	14.67± 0.57[b]	17.67±1.155[b]
I. batatas	7.20± 4.10[b]	17.00± 1.73[b]	19.00± 1.00[b]

Mean values (mean+standard p_0.05) represent error of feeding holes (cumulative) by sweet potato weevil on different *Ipomoea* sp. leaves

Table 2. Vine feeding (no. of holes) by the weevils on *Ipomoea* species in the multiple choice bioassay

Species	1st Day	2nd Day	3rd Day
I. mauritiana	7.67± 2.96^{ab}	10.56± 3.37^{ab}	14.89± 2.14^{b}
I. triloba	4.22 ± 0.50^{b}	9.56 ± 1.38^{b}	12.00 ± 1.19^{b}
I. palmata	12.67 ±3.18 ^{a}	18.00 ±3.46 ^{a}	26.44±1.01^{a}
I. obscura	8.78 ± 1.50^{ab}	14.22± 3.65^{ab}	16.44 ± 2.14^{b}
I. batatas	11.22± 1.16^{a}	17.78± 2.41^{a}	24.22± 2.79^{a}

Mean values (mean+standard, *p*_0.05) represent the feeding holes (cumulative) by sweet potato weevil on different *Ipomoea* sp vines

Table 3. Tuber feeding (no. of holes) by the weevils on *Ipomoea* species in the multiple choice bioassay

Species	1st Day	2nd Day	3rd Day
I. mauritiana	12.67 ± 2.30^{a}	22.67± 6.02^{a}	30.33 ± 2.51^{a}
I. triloba	10.67 ± 3.05^{a}	22.67 ± 8.73^{a}	34.33± 4.93^{a}
I. palmata	12.00 ± 0.00^{a}	26.67± 10.40^{a}	31.33± 5.50^{a}
I. obscura	12.67 ± 1.15^{a}	27.33± 3.51^{a}	30.67 ± 3.21^{a}
I. batatas	19.00 ± 2.64^{b}	31.00± 1.00^{a}	37.33± 2.08^{a}

Mean values (mean+standard, *p*_0.05) represent the feeding holes (cumulative) by sweet potato weevil on different *Ipomoea* sp roots

Table 4. Weevil feeding (no. of holes) on *Ipomoea* species in the two choice bioassay

Species	1st Day	2nd Day	3rd Day
Leaves			
I. mauritiana	5.67±2.51	8.67±1.15	13.00±1.00
I. batatas	16.00±2.00	18.33±1.52	26.67±2.88
Vines			
I. mauritiana	11.00±1.00	13.00±1.73	14.67±2.51
I. batatas	16.33±0.57	19.00±2.64	25.67±1.15
Tubers			
I. mauritiana	6.33±0.57	12.33±2.51	17.67±1.52
I. batatas	28.33±7.63	35.00±5.00	43.00±4.58

Mean values (mean+standard, *p*_0.05) represent the feeding holes (cumulative) by the weevil

Gas chromatography-Mass spectrum analysis:

In all the multiple choice as well as two choice bioassay the feeding of weevils was significantly less in *I. mauritiana* which may be due to the presence of various phytochemical constituents. This shows the non-preference of the weevils always depends on the nature of host plant. GC-MS analysis of methanol extract of samples revealed phytochemical compounds, its retention time (RT) and peak area (%). The bioactivity of the identified compounds reported are presented along with its reference (Table 5). The phytochemical constituent of *I. mauritiana* include compounds undecane and quinic acid which are reported to have insecticidal activity whereas sucrose reported to enhance insecticidal activity**.** The most prevailing compounds identified in *I. mauritiana* were sucrose (77.01%), quinic acid (20.93%) whereas in *I. batatas* they were melezitose (38.53%) and alpha-I-rhamnopyranose (21.26%).

Higher levels of octadecyl and hexadecyl esters of hydroxycinnamic acids were identified in the root surface and root latex of sub-saharan sweetpotato variety, New Kawogo, contributing resistance to sweet potato weevil (Stevenson *et al.,* 2009). Anyanga *et al.* (2013) reported that the these compounds in high concentrations on root surfaces was strongly associated with resistance against adult oviposition and feeding. They reduce the development of sweet potato weevil larvae and suggested that differences in the concentration of these compounds between varieties explain differences in resistance. Among the five *Ipomoea* species the weevil infestation was significantly less in *I. mauritiana*. Phytochemical screening of methanolic extract revealed the presence of various compounds which are reported to have insecticidal activity. These components might be responsible for the low weevil infestation in *I. mauritiana.*

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Table 5: List of the phytochemical compounds detected from the methanol extract of *Ipomoea* species through GC–MS analysis

No	R/T	Peak %	Compound	Bioactivity	Reference
I. mauritiana					
1.	6.15	2.06	Undecane *Ludwigia stolonifera*	Constituent of	Baky *et al.,* 2021
2.	14.08	77.01	Sucrose	Insecticide activity	Ezhilan and Neelamegam, 2012
3.	17.97	20.93	Quinic acid	Insecticidal activity	Li *et al.,* 2021
I. batatas					
1.	5.68	3.48	D-Alanine, N-proparglyoxy carbonyl-decyl ester	Constituent of *Averrhoa bilimbi*	Suluvoy and Grace *et al.,* 2017
2.	6.85	2.26	DL-Arabinose	Antimicrobial activity	Mohammed *et al.,* 2018
3.	6.92	2.04	2-Deoxy-2-fluoro-1,6-anhydro -β-d-glucopyranose	Constituent of *Alternaria alternata*	Kamal *et al.,* 2015
4.	7.13	2.03	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	Antifungal activity	Teoh and Don, 2015
5.	9.07	3.28	5-Hydroxymethylfurfural	Insecticidal activity	Chuang *et al.,* 2018
6.	9.66	7.03	5-O-Methyl-d-gluconic acid dimethylamide	Antimicrobial, antioxidant	Kazi and Gude, 2022
7.	10.24	2.19	Octanamide, N-(2-mercaptoethyl)	Secondary metabolite of *Vitis vinifera*	Kadhim *et al.,* 2017
8.	12.40	2.77	Methyl 4-nitrohexanoate	Constituent of *Hugonia mystax*	Vasuki *et al.,* 2022
9.	14.60	38.53	Melezitose	Insecticidal activity	Gore and Schal *et al.,* 2004
10.	18.45	4.04	Desulphosinigrin	Antibacterial activity	Olajuyigbe *et al.,* 2018
11.	26.38	1.97	1H-Benzocyclohepten-7-ol, 2,3,,4,4a, 5,6,7,8-octahydro-1,1,4a	Floral volatile constituents of *Crataeva religiosa*	Sharma *et al*., 2018
12.	27.04	1.83	Spiro[4,5]decan-7-one,1,8 -dimethyl-8,9-epoxy-4-isopropyl	Anti-inflammatory	Subin and Jagathy 2017
13.	28.22	0.74	Santamarine	Natural antioxidant with anti-photoaging	Oh *et al.,* 2021
I. palmata					
14.	6.48	1.28	Maltol	Mosquito larvicidal activity	Rajamanikyam *et al.,* 2017
15.	9.41	1.28	4-Methylmannitol	Constituent of khat leaves	Alsanosy *et al.,* 2020
16.	10.53	1.77	2H-Pyran-2-onetetrahy dro-6-propyl-	Fatty acid composition of *Trichosanthes cucumerina* bio-oil	Manimaran *et al.,* 2020

17.	13.88	3.29	Panaxydol	first isolated from roots of P. ginseng induces apoptosisin cancer cells	Takahashi *et al.,* 1964; Kim *et al.,* 2016
18.	14.00	2.56	Melezitose	Insecticidal activity	Gore and Schal *et al.,* 2004
19.	18.07	7.65	Quinic acid	Insecticidal activity	Li *et al.,* 2021
20.	23.39	2.33	3-(6,6-Dimethyl-5-oxohept-2-enyl)-cycloheptanone	Constituent of *Myoporum bontioides*	Minh et al., 2020
21.	26.56	5.22	Scopoletin	Antitermite activity	Adfa *et al*., 2010
22.	27.04	2.59	1,8-Naphthalenedione, 8a-ethylperhydro	Cconstituent of *Plectranthus hadiensis*	Sripathi *et al.,* 2017

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