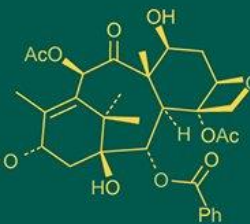
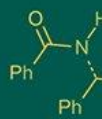


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Bioprospecting of sweet potato (*Ipomoea batatas*) vines for the management of *Cylas formicarius* (Sweet potato weevil)

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Abstract

The present study aims to develop an effective management strategy against the major pest of sweet potato (*Ipomoea batatas*), the sweet potato weevil (*Cylas formicarius* Boheman) (Coleoptera: Curculionidae), through the isolation of kairomones from host plant vines. The volatile chemical profile of sweet potato was examined in relation to the host-finding behaviour of *C. formicarius*. Among the extraction methods evaluated, cold extraction yielded both qualitative and quantitative advantages in recovering volatile compounds from fresh vines. The acetone crude extract obtained through this technique was subsequently fractionated using automated silica gel column chromatography (ACC) with a series of solvents. Both crude and purified fractions were assessed for the olfactory response of *C. formicarius* under laboratory and field conditions. Gas chromatography-mass spectrometry-electroantennogram (GC-MS-EAG) analysis of the acetone fractions identified eight compounds eliciting significant antennal responses in the weevil. Among these, 1,3-nonanediol, L-Gala-L-ido-octose, and D-Glycero-D-ido-heptose exhibited responses analogous to female sex pheromones. Additionally, five compounds; 3,4-hexanediol, 2,5-dimethyl; (S)-4-amino-2-benzylbutanoic acid; 9-acetoxy nonanal; 5-t-butyl-6-methyl-hept-3-ene-2,5-diol; and 6-acetyl-β-D-mannose; showed sex-neutral attraction, indicating their potential role as kairomones for population management of *C. formicarius*. No compound with female-specific activity was detected in the present study.

Keywords: Semiochemical response, sweet potato kairomone, bio control measure, kairomone trap, solvent extraction technique, electro antennogram study

Introduction

Detrimental effects of synthetic insecticides on health, environment and organisms have necessitated the exploration of alternative strategies for insect control. The insect attractants represent an important link within these systems. Semiochemicals play an important regulative role in the life of insects in addition to other communication channels as optic, acoustic, or tactile communications (Howse *et al.*, 1998; El-Shafie and Faleiro, 2017; Annie *et al.*, 2017) [9, 27]. In the field of sensory ecology, semiochemicals (info-chemicals) are classified as pheromones (intra specific) and allelochemicals (inter specific); the latter of which include kairomones (benefit the receiver), allomones (benefit the sender), and synomones (benefit both) (Dusenbery, 1992; Annie *et al.*, 2017) [8]. Biologically active volatile phytochemicals represent an important means of communication within plants and between plants and other organisms in their environment (Hans, 1995). Phytochemicals also play critical roles in insect behaviour, modulating host finding, feeding, and oviposition responses for certain species where they may function as attractants, repellents, feeding and oviposition stimulants, deterrents, and toxicants (Tumlinson, 1988; Metcalf and Metcalf, 1992) [23, 40]. Phytochemicals play critical roles in facilitating the development of host-plant resistance in long-run (Peshin and Dhawan, 2009) [28].

Sweet potato is the seventh most important crop in the world with a total production of 92 million metric tonnes in the year 2018 (FAO, 2019). Despite the crop's economic importance, widespread sweet potato weevil infestation results in losses of millions of dollars annually (Talekar, 1994) [39]. By being a major staple crop, sweet potato enjoys a wide literature support in its cultivation practice. (Hue and Low, 2015) [16] summarise most of the management Measures on the crop loss by *C. formicarius*.

Even in this context, farmers are in big menace with this weevil attack. The level of infestation in the different sweet potato genotypes was reported to be related to the concentration of kairomones in the periderm of the tubers (Son *et al.*, 1990) [35]. For example, Boehmeryl acetate-a kairomone identified in sweet potato tuber surface acts as an ovipositional stimulant for female weevils (Son *et al.*, 1990) [35]. The present study aims at the extraction and isolation of phytochemicals from sweet potato vines for sweet potato weevil management.

Materials and Methods

1. Extraction techniques used for the isolation of kairomones from sweet potato

Sweet potatoes were grown in the year 2018 at the ICAR-Central tuber crops research institute (ICAR-CTCRI) using standard sweet potato production practices (Granberry and Kelley, 1987) [12]. Following techniques were employed for the extraction of possible kairomones from sweet potato (*Ipomoea batatas* Var. *Sree Arun*). All extractions were done from sweet potato plants maintained in an insect free condition (poly house) for eliminating the possible involvement of pheromones by the natural weevil population.

a. Solvent extraction from dried vines

Vines of fresh sweet potato were thoroughly cleaned and dried in hot air oven maintained at 50 °C for three days. It was then coarsely powdered using a conventional mixer grinder. It weighed approximately 450g. The powdered material was subjected to repeated extraction three times with hexane, acetone, methanol and double distilled water (dd_water) (Make: Merk) (500 ml × 3) at room temperature. The extracts were then concentrated under reduced pressure using a Buchi R-200 rotary evaporator (Buchi, Labortechnik, Switzerland).

b. Solvent extraction from fresh vines

Fresh vines of sweet potato, chopped in one cm length were used for repeated extraction three times with hexane, acetone, methanol and dd_water (Make: Merk) (500 ml × 3) at room temperature (30±2 °C). After each extraction, the solvent was removed by vacuum evaporation so as to obtain the crude extract in a concentrated form by using a Buchi R-200 rotary evaporator.

c. Cold extraction from fresh vines

For the study an ideal solvent with a mid-polarity need to be ascertained so that one can assure representative chemical species from both the ends of polarity scale. Fresh sweet potato vein cuttings (250 g) were kept in acetone (medium polar solution-PI=5.1) for three days (500 ml × 3 at 25 °C) and was cold extracted. After extraction, the solvent was removed by using a lyophilized (iL Shin Bio Base TF05503 freeze dryer) so as to obtain the crude extract in a concentrated form. The crude extract was then analyzed by gas chromatography-mass spectrometry (GC-MS) for compound profiling.

d. Purification and fractionation of crude extracts by automated column-chromatography (acc)

The packing material silica gel (60-120 mesh size) is slurried with hexane and poured in column (30cm long and 2cm dia.) to get a homogeneous packing of the silica gel.

Application of Acetone concentrated crude extracts (10 ml), from all the three extraction technique were employed for further fractionation under ACC. When the respective crude extract has been absorbed on the top of the column, the space above it is filled with the first eluent (hexane~50 ml) with no time for air entrainment in the silica. From the column run, the fractions thus obtained are collected in separate vials with a fixed time interval of 5 minutes using the time automation. Elution started using hexane and was followed by different solvents such as diethyl ether, dichloro methane, acetone, ethanol, methanol and dd_water. Different eluents (50 ml) were continuously poured sequentially according to increasing order of their polarity and respective fractions were separated. There were two fractions for each solvent and were named as respective solvent fraction A and B.

Throughout the experiment the fractions were flash evaporated or lyophilised until all the solvent evaporates or to a minimum quantity of 0.25 ml. All the liquid fractions isolated using the above given techniques were stored in-20 °C in air-tight 10 ml vials (Make: Tarson).

2. Rearing of *Cylas formicarius*

Infested sweet potato tubers were collected from ICAR-CTCRI field and were maintained in bottles with spread muslin cloth as lid to ensure enough ventilation. We observed maximum emergence in 10±2 days. All the freshly emerged weevils in this period were reared in another set of well-ventilated bottles (rearing bottles) with uninfested tuber as food source (65 nos. per bottle; with 1:1 sex ratio) at 30 °C and 70±5 RH.

3. Bio activity of kairomones from sweet potato

a. Olfactory studies

Both the crude liquid fractions (isolated by the method of solvent extraction from dried vines, solvent extraction from fresh vines & cold extraction from fresh vines) and the purified fractions from all the three extraction techniques were screened for olfactory response on *C. formicarius*. 'Y' shaped lactometer is used for the study (Fig. 1). From the crude fraction, 10 µl, 50 µl, 75 µl, 100 µl and from the purified fraction, 75 µl were used. Different fractions were applied in cotton balls (1g) and were kept on one of the two sample inlets, and the other inlet was considered as control with cotton soaked with dd_water. In extracts, were solvent fraction suspected even after the maximum possible flash evaporation, respective pure solvents were used as control. At time zero of the experiment, 52 weevils (26 nos. of male: 26 nos. of female) were released in the insect release chamber and were continuously monitored for their preference towards the fraction holding chamber (test). Numbers of weevils responded positively towards the tests and the average time taken were calculated for each sample. All experiments were replicated thrice. After each sample screening, the lactometer were assured to wash properly so that no residue will interfere the subsequent screening. For every trial fresh batches of weevils (from different rearing bottles) were used to eliminate any possible replication bias. Effective response of male and female weevils can be calculated by using the formula;
Effective response (ER) value of male or female weevil =
No. of male or female weevil preferring test-No. of male or female weevil preferring control

b. Field studies

In the field (10 × 20 m isolated fields of sweet potato) three replication traps (with same test extract- T_1 , T_2 & T_3) and one positive control (PC) trap with sex pheromone lure of *C. formicarius* (Make: Pherobio) were kept on all the four corners (Fig. 2). Attractions of weevil towards the traps were monitored for different time periods (1h, 2h, 5h, 8h, 12h and 24h). Every distinct extracts were tested in different such sweet potato fields of ICAR-CTCRI, Thiruvananthapuram (8.5241°N, 76.9366°E) on climatically compatible days. All the weevils collected in traps were preserved separately as batches in 70% ethanol and screened for male, female ratio.

4. Molecular characterization of acetone extract from sweet potato

a. Gas chromatography (gc)-mass spectrometry (ms)

Gas chromatogram was generated for the acetone crude fraction by using cold extraction technique as suggested by Wang and Kays (2002) [45]. Gas chromatography (HP6890; Agilent Technologies USA Ltd) directly linked to a HP5973 mass selective detector (Agilent Technologies) operated in electron impact mode (source temperature 230 °C; transfer line 250 °C). The HP-5 MS phenyl methyl siloxane non polar capillary column (0.25 mm × 30 m × 0.25 μ .) at a maximum temperature of 350 °C (Agilent part No 190915-433) was used for the separation of fractions. The mobile phase was helium with very high purity (Praxair India Ltd.) and was passed through the universal trap for removing the contaminants. The split inlet was used with split ratio of 50:1 and inlet temperature of 280 °C. The oven temperature program was set at 70 °C with 2 minutes hold and a ramp of 10 °C min⁻¹ till 260 °C and held for 5 min with column flow of 1 ml/min. The mass spectral detector was maintained at a temperature of 280 °C with the interface temperature of 230 °C. The mass spectra created using the MS was compared with the Wiley mass spectral library powered by Wiley, 2012 and NIST, 2012 version.

b. Gas chromatography-mass spectrometry coupled with electro antennogram analysis (gc-ms-ms-eag)

Fractions that show high bioactivity both in laboratory and in field trial were recorded on the extirpated antennae of both sexes of 30 day-old virgin *C. formicarius*. Electroantennogram (EAG) recordings were made with Ag-AgCl glass microelectrodes filled with ringer solution (7.2 g NaCl, 0.37 g KCl, 0.17 g CaCl₂, pH 7.3-7.4 in one litre water). The weevils were anaesthetized by chilling (at -20 °C for 1 minute and 40 seconds), and its antennae were excised from the head with a microscalpel. It was inserted into the electrode and signals were passed through a high impedance amplifier and analyzed using a customized software package (Syntech). In the coupled GC-MS-MS-EAG system, the effluent from the GC column is directly passed through the antennal preparation. Separation of volatiles was achieved in a GC (Agilent Technologies, 6890N) equipped with a cold on-column injector, a flame ionization detector (FID), and MS-MS (TQ 8030 SHIMADZU instrument & Syntech instrument) using an HP-1 column (50 m, 0.32 mm i.d. 0.52 μ m f.t.). The oven was maintained at 30 °C for 2 min. and then programmed for increments at 5 °C min⁻¹ to 250 °C. The carrier gas was helium. Outputs from the EAG amplifier and the FID were monitored simultaneously and analyzed using the MS-MS software package. Peaks eluting

from the GC column were considered active if they elicited EAG response in three or more coupled runs. Authentic standards were tested using a delivery system which employed a filter paper strip in a disposable Pasteur pipette cartridge (Wad hams *et al.*, 1982) [42]. The stimulus (2 s duration) was delivered into a purified airstream (0.5l/min) flowing continuously over the preparation using a stimulus controller (Syntech CS02). Samples (10 μ l) of the standard solution of test compounds (1 mg/ml in redistilled methanol) were applied in the cartridge. Acetone (10 μ l) was given for the control stimulus. Five replicates were performed for each sample using antennae from 5 different weevils. GC-MS-MS-EAG responses of both male and female *C. formicarius* were recorded.

5. Analysis

All statistical analyses were conducted using the software R Studio, version 3.3.2 (58) and by Microsoft Excel 2010. Names of statistical tests and levels of significance used are given in the appropriate figure legends. In general, the Duncan's Multiple Range Test ($P < 0.05$) was used for the comparative analysis and for the significance studies. For the effective response comparison studies, trend lines were drawn and linear equations were generated since the response can be an expected continuum for further higher doses. Proportion of the variance were also provided in the legends.

Results and Observations

Solvent extraction from dried vines resulted, 0.75 ml of crude hexane extract, 1.25 ml of crude acetone extract, 2 ml of crude methanol extract and finally 3.25 ml of crude water extract. Bioactivity study on the olfactory response of both male and female *C. formicarius* over the crude fractions isolated by solvent extraction from dried vines shows high effective response (ER) values in highly polar fractions (Figure 3).

By using the method of solvent extraction from fresh vines, 2.25 ml of crude hexane extract, 6 ml of acetone extract, 4.5 ml of methanol extract and 7 ml of water extract were obtained. Olfactory response of *C. formicarius* over the crude fractions by solvent extraction method from fresh vines shows an ER value of 16.7 by the male weevil for the acetone extract at a dose of 100 μ l and the highest female ER value of 12.7 at a dose of 100 μ l. (Figure 4).

Cold extraction from fresh vines resulted, 1.75 ml of crude hexane extract, 3.25 ml of crude acetone extract, 4.25 ml of crude methanol extract and 5.50 ml of crude water extract. Study on olfactory response of the weevil over the crude fractions isolated by cold extraction from fresh vines shows the highest effective response value for male weevils as 18.7 (Figure 5).

After the fractionation through automated column chromatography a total of 14 fractions were result out when 7 different solvents were employed. All the fractions were scrutinized under bio assay for olfactory response of both male and female *C. formicarius* and the following results were obtained and were described in table 1.

From the field trials total numbers of *C. formicarius* collected in response to different crude extracts over different time periods were represented in figure 6. Positive control yields the highest number of weevils (203 $\pm$ 33.8 nos) on trap at the second hour of the experiment. Crude acetone extract from fresh veins at a dose of 100 μ l shows the best

result among test extracts by trapping 39 ± 7.5 weevils with a time period of 24 hrs. (Figure 6). Crude extract profile (Figure 7) was generated for the acetone fraction from the cold extraction technique using gas chromatography and mass spectroscopy. Twenty four major compounds were detected, of which six are reported to have kairomonal

activity (Table 2). From GC-MS-MS-EAG (Figure 8), five different compounds show clear response or stimuli in the antennae of *C. formicarius* irrespective of sex and three different compounds were observed as specific attractant to male weevils. No specific compound was screened with specific attraction to female weevils (Table 2).

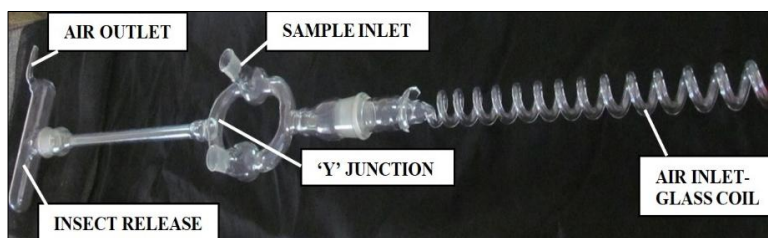


Fig 1: 'Y' shaped olfactometer used for the olfactometric study on *Cylas formicarius*

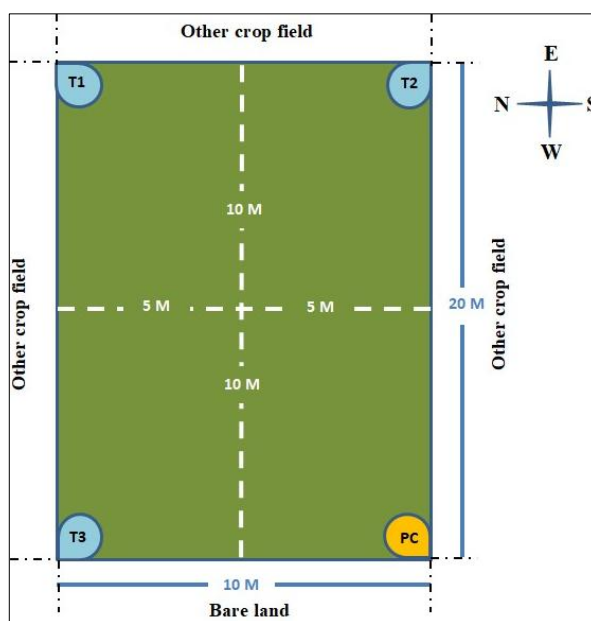
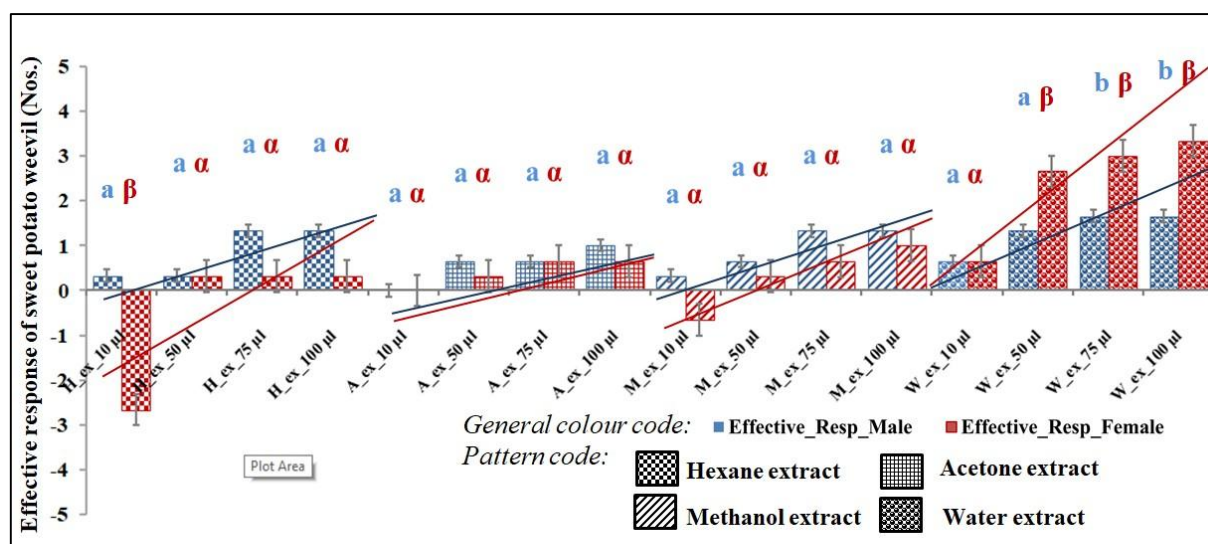
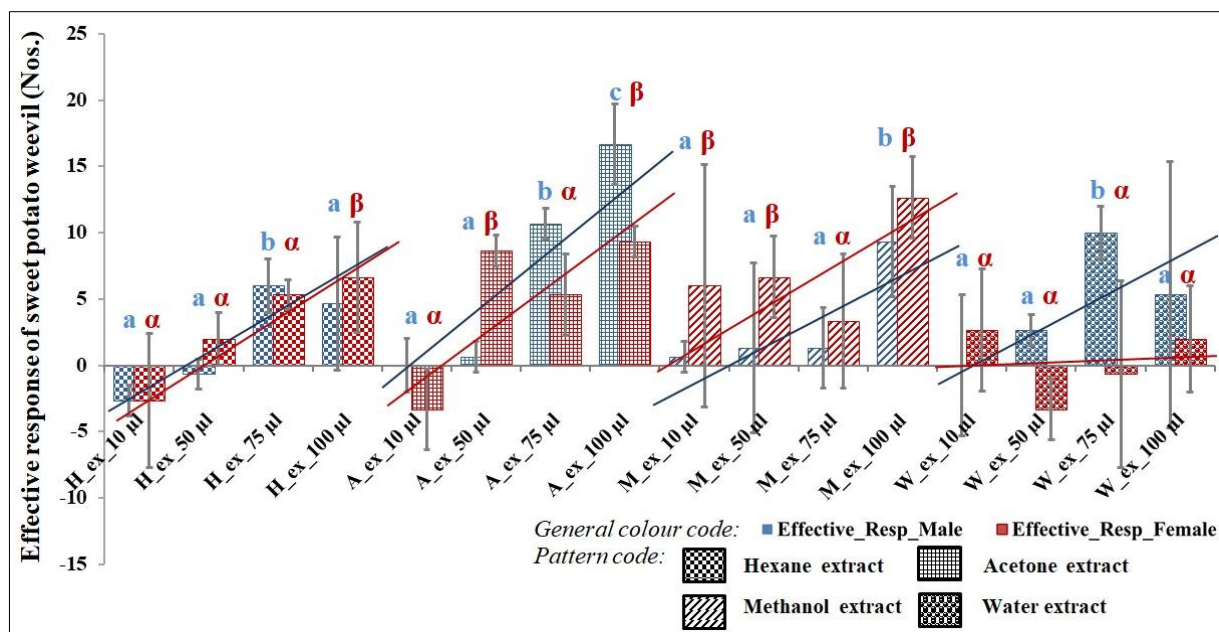


Fig 2: General field sketch of treatment plot employed in the study [T₁: Test-1, T₂: Test-2, T₃: Test-3 and PC: positive control]



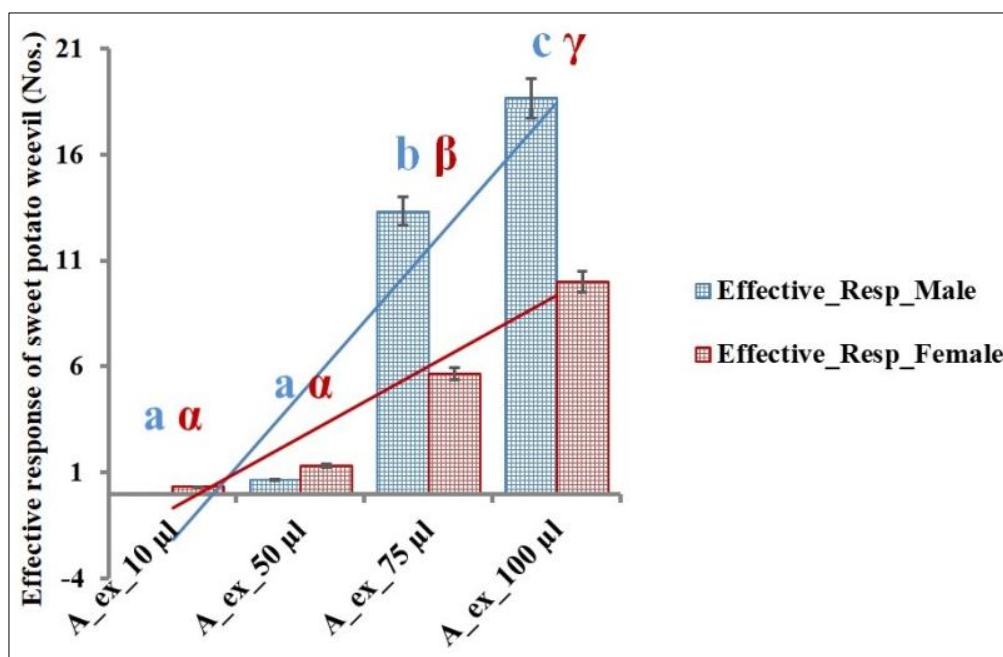
The Duncan's Multiple Range Test ($p < 0.05$) was used and the significant differences are indicated by different English and Greek letters. (rep. 10, Mean $\pm$ SD) Linear equation for effective response by male (ERM) to Hexane extract (H_ex): $y = 0.4x - 0.17$, $R^2 = 0.8$; effective response to female (ERF) to Hexane extract: $y = 0.897x - 2.66$, $R^2 = 0.6$; ERM to Acetone extract (A_ex): $y = 0.3x - 0.17$, $R^2 = 0.8562$; ERF to Acetone extract: $y = 0.231x - 0.165$, $R^2 = 0.8909$; ERM to Methanol extract (M_ex): $y = 0.367x - 0.005$, $R^2 = 0.8959$; ERF to Methanol extract: $y = 0.531x - 0.995$, $R^2 = 0.9167$; ERM to Water extract (W_ex): $y = 0.333x + 0.495$, $R^2 = 0.8317$; ERF to Water extract: $y = 0.835x + 0.325$, $R^2 = 0.8071$

Fig 3: Effective response of sweet potato weevil to various solvent extracts from dried vines



The Duncan's Multiple Range Test ($p < 0.05$) was used and the significant differences are indicated by different English and Greek letters. (rep. 10, Mean $\pm$ SD) Linear equation for Effective response by male (ERM) to Hexane extract (H_ex_): $y = 2.862x - 5.32$, $R^2 = 0.7913$; Effective response to female (ERF) to Hexane extract: $y = 3.129x - 4.99$, $R^2 = 0.946$; ERM to Acetone extract (A_ex_): $y = 5.998x - 8$, $R^2 = 0.9182$; ERF to Acetone extract: $y = 3.465x - 3.665$, $R^2 = 0.5906$; ERM to Methanol extract (M_ex_): $y = 2.601x - 3.34$, $R^2 = 0.663$; ERF to Methanol extract: $y = 1.665x + 3$, $R^2 = 0.298$; ERM to Water extract (W_ex_): $y = 2.333x - 1.335$, $R^2 = 0.4987$; ERF to Water extract: $y = 0.069x - 0.005$, $R^2 = 0.0011$

Fig 4: Effective response of sweet potato weevil to various solvent extracts from fresh vines



The Duncan's Multiple Range Test ($p < 0.05$) was used and the significant differences are indicated by different English and Greek letters. (rep. 10, Mean $\pm$ SD) Linear equation for Effective response by male (ERM) to Acetone extract (A_ex_): $y = 6.865x - 9$, $R^2 = 0.907$; ERF to Acetone extract: $y = 3.334x - 4.005$, $R^2 = 0.9433$

Fig 5: Effective response of sweet potato weevil to acetone extracts from cold extraction technique on fresh vines

Table 1: Effective response of sweet potato weevil to purified fractions of acetone crude extract isolated by various Extraction techniques.

Extraction techniques.					
Sl. No.	Sample name	Sample yield from 10 ml crude	Sample Con.	Effective response of male <i>C. formicarius</i> (ERM)	Effective response of female <i>C. formicarius</i> (ERF)
Solvent extraction from dried vines>> acetone crude fraction					
1	Hexane extract A	0.25 ml	75 µl	-2±1.33	-4±0.54
2	Hexane extract B	0.25 ml		-1±2.23	-2±0.66
3	Diethyl ether extract A	0.25 ml		3±0.54	-1±0.26
4	Diethyl ether extract B	0.25 ml		-2±1.15	0±1.33
5	Dichloro methane extract A	0.25 ml		2±1.25	2±1.24
6	Dichloro methane extract B	0.25 ml		1±0.56	-8±1.56
7	Acetone extract A	1.75 ml		4±1.42	5±2.23
8	Acetone extract B	1.25 ml		3±1.24	2±0.66
9	Ethanol extract A	0.75 ml		3±0.56	2±1.24
10	Ethanol extract B	0.50 ml		5±1.24	2±1.56
11	Methanol extract A	0.75 ml		2±0.33	1±1.24
12	Methanol extract B	0.75 ml		4±1.24	3±1.33
13	dd. Water extract A	1.25 ml		6±1.56	2±0.56
14	dd. Water extract B	0.75 ml		3±0.54	1±0.33
Solvent extraction from fresh vines>> acetone crude fraction					
1	Hexane extract A	0.25 ml	75 µl	2±2.33	1±1.56
2	Hexane extract B	0.25 ml		-4±1.24	-3±0.24
3	Diethyl ether extract A	0.25 ml		4±0.66	2±2.33
4	Diethyl ether extract B	0.25 ml		2±1.56	-6±1.56
5	Dichloro methane extract A	0.25 ml		2±1.33	2±2.33
6	Dichloro methane extract B	0.25 ml		-6±4.24	1±1.56
7	Acetone extract A	2.00 ml		4±1.24	2±0.54
8	Acetone extract B	1.50 ml		4±1.33	2±0.56
9	Ethanol extract A	1.25 ml		8±2.66	6±3.33
10	Ethanol extract B	0.75 ml		6±2.33	3±2.66
11	Methanol extract A	1.00 ml		3±0.54	2±0.56
12	Methanol extract B	0.75 ml		7±1.56	3±0.24
13	dd. Water extract A	1.25 ml		12±6.24	5±2.24
14	dd. Water extract B	0.75 ml		8±2.33	6±3.54
Cold extraction from fresh vines>> acetone crude fraction					
1	Hexane extract A	0.25 ml	75 µl	4±0.66	2±0.33
2	Hexane extract B	0.25 ml		1±0.24	3±1.24
3	Diethyl ether extract A	0.25 ml		-2±1.24	2±2.66
4	Diethyl ether extract B	0.25 ml		2±0.54	-4±0.33
5	Dichloro methane extract A	0.25 ml		3±1.26	-3±1.66
6	Dichloro methane extract B	0.25 ml		6±2.66	2±1.56
7	Acetone extract A	1.25 ml		8±3.33	4±2.54
8	Acetone extract B	1.00 ml		4±0.66	4±0.66
9	Ethanol extract A	0.75 ml		3±1.56	4±1.56
10	Ethanol extract B	0.75 ml		11±5.33	6±2.66
11	Methanol extract A	0.75 ml		14±4.66	8±4.56
12	Methanol extract B	0.50 ml		9±3.33	6±3.24
13	dd. Water extract A	0.75 ml		17±3.56	10±4.26
14	dd. Water extract B	0.25 ml		15±4.24	9±3.33
Colour code representations		ERV ≥ + 22 (85% desirability or more) indicates the status of a strong attractant			
		+ 22 > ERV ≥ + 16 (62% desirability or more upto 85%) indicates the status of a moderate attractant			
		+ 16 > ERV ≥ + 10 (38% desirability or more upto 62%) indicates the status of a mild attractant			
		ERV ≤-22 (85% repellency or more) indicates the status of a strong repellent			
		-22 < ERV ≤-16 (62% repellency or more upto 85%) indicates the status of a moderate repellent			
		-16 < ERV ≤-10 (38% repellency or more upto 62%) indicates the status of a mild repellent			
		-10 <ERV> + 10 were considered as no response			
Con: Concentration; dd: double distilled;>>: Specific continuum					

[A: Crude acetone extract from dried vines_solvent extraction technique; B: Crude acetone extract from fresh vines_solvent extraction technique; C: Crude acetone extract from fresh vines_cold extraction technique; D: Purified acetone extract-A from dried vines_solvent extraction technique; E: Purified acetone extract-B from dried vines_solvent extraction technique; F: Purified acetone extract-A from fresh vines_solvent extraction technique; G: Purified acetone extract-B from fresh vines_solvent extraction technique; H: Purified acetone extract-A from fresh vines_cold extraction technique; I: Purified acetone extract-B from fresh vines_cold extraction technique; P: Positive control]

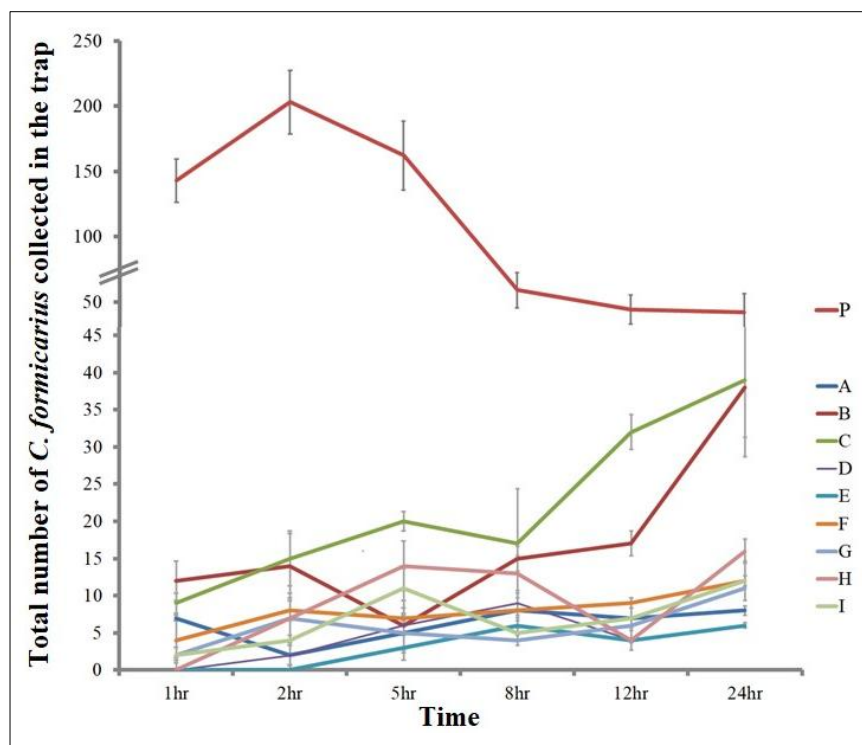


Fig 6: Field level response of sweet potato weevil to both crude as well as purified fractions of acetone extract isolated by various extraction techniques

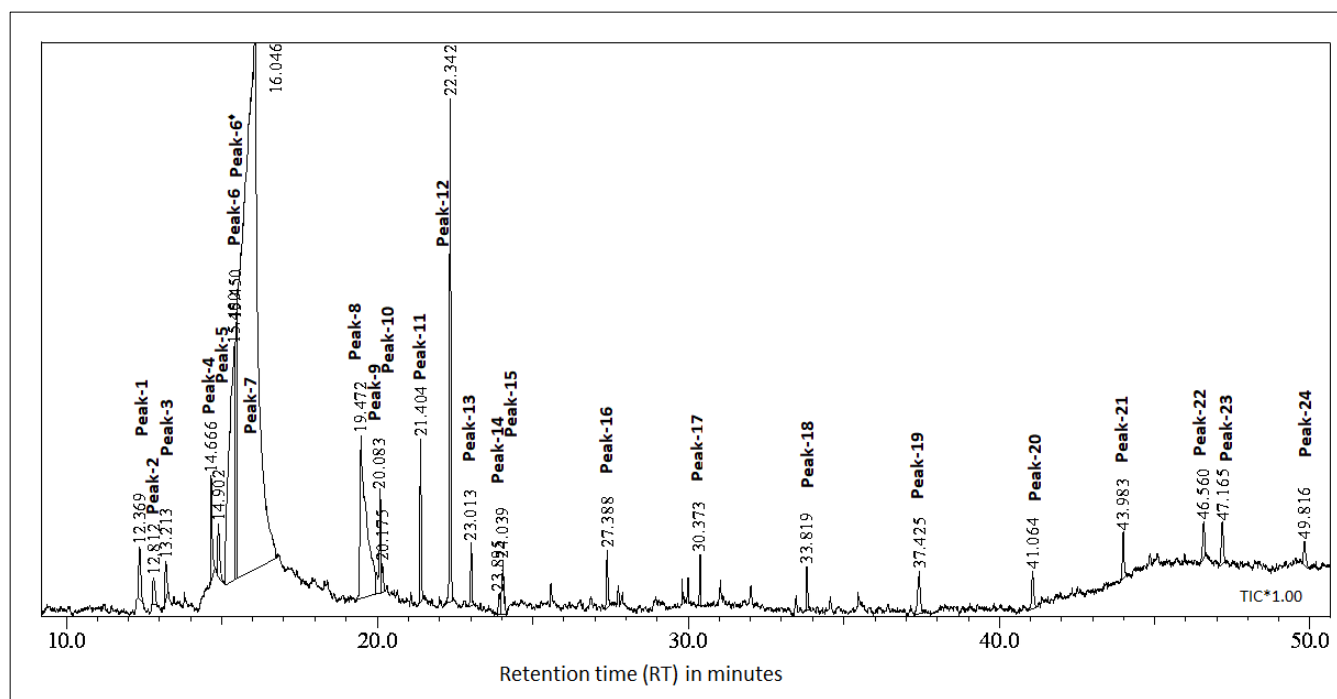


Fig 7: Gas chromatogram of crude acetone extract from cold extraction technique of fresh sweet potato vines

Table 2: Compounds identified through mass spectroscopy of crude acetone extract from cold extraction technique of fresh sweet potato vines

Peak No	RT	Compound identified by GC-MS
1	12.37	1,3-nonanediol
2	12.81	5-t-Butyl-6-methyl-hept-3-ene-2,5-diol
3	13.21	3-octanol
4	14.67	2,3,5,6 diacetone 4-O-methyl mannitol
5	14.90	2-methyl butane-1,2,3,4 tetraol
6	15.10	9-Acetoxy nonanal
6*	15.15	9-Acetoxy nonanal*
7	16.05	2-butyl dimethyl Siloxy butane
8	19.47	5-O-methyl d-gluconic acid dimethyl amide
9	20.08	3-hydroxy norvaline
10	20.18	2-hydroxy,4-methyl benzaldehyde
11	21.40	7,7 bis methyl thio 1-(2 furyl) 2,4,6 heptatriene 1-one
12	22.34	3,4-Hexanediol, 2,5-dimethyl
13	23.01	1, 8-di bromo octane
14	23.90	1-bromo do decane
15	24.04	Benzene dicarboxylic acid diethyl ether
16	27.39	Hexadecamethyl cyclooctasiloxane
17	30.37	Phenylacetaldehyde
18	33.82	Methyl geranate
19	37.43	(S)-4-Amino-2-benzylbutanoic acid
20	41.06	Nerolidol iso butyrate
21	43.98	6-Acetyl-.beta.-d-mannose
22	46.56	d-Glycero-d-ido-heptose
23	47.17	l-Gala-l-ido-octose
24	49.82	2-phenyl 3-iodo 5-nitrothiophene

RT; Retention time in minutes, *Peak overlap within limited MS detection,

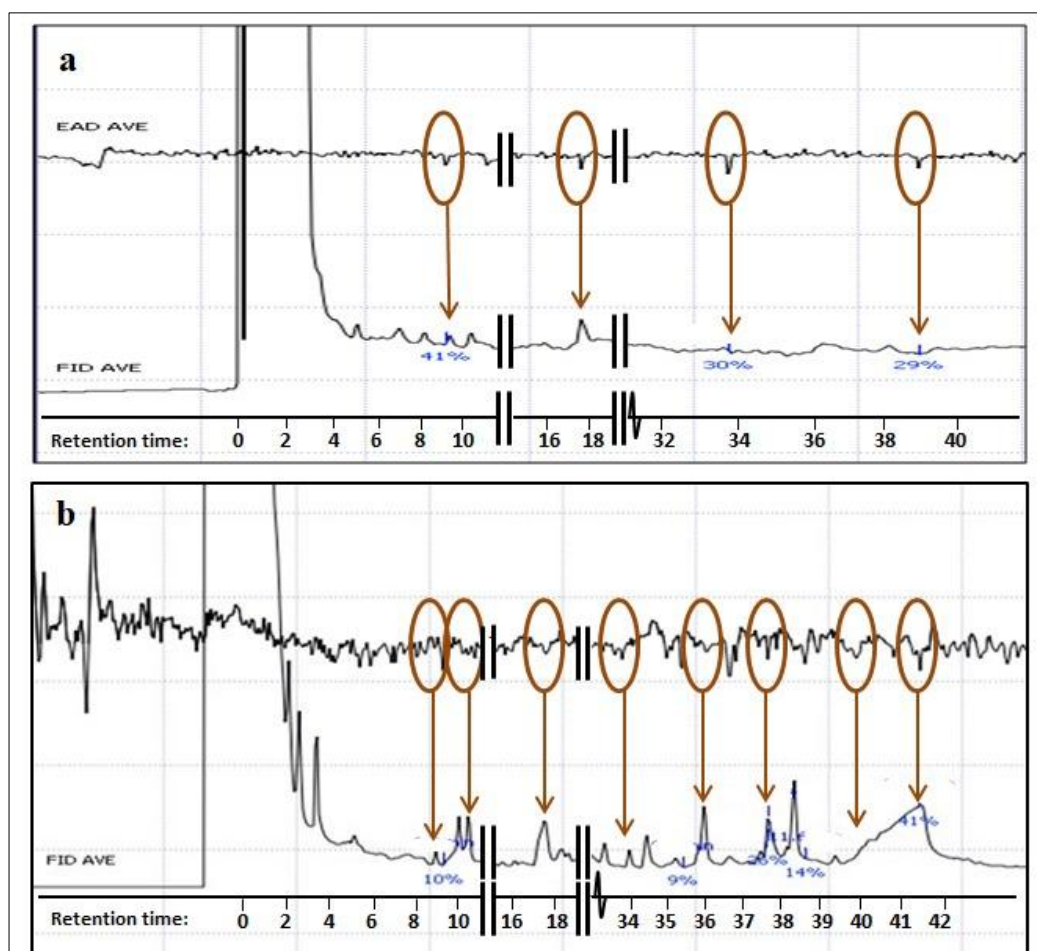
**Fig 8:** Electroantennographic Detection (EAD) Responses of *Cylas formicarius* to Volatile Compounds Isolated from Sweet Potato

Table 2: Compounds that show clear positive stimulation in GC-EAG-MS on the antennae of *Cylas formicarius*

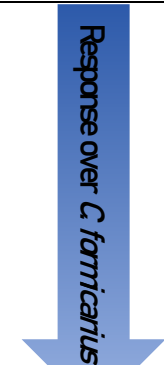
Male Female				
RT.	Compound		RT.	Compound
10.73	9-Acetoxynonanal		17.97	3,4-Hexanediol, 2,5-dimethyl
8	(+ -)-1,3-nonanediol		33.06	(S)-4-Amino-2-benzylbutanoic acid
8.44	5-t-Butyl-6-methyl-hept-3-ene-2,5-diol		10.73	9-Acetoxynonanal
42.8	l-Gala-l-ido-octose		8.44	5-t-Butyl-6-methyl-hept-3-ene-2,5-diol
33.06	(S)-4-Amino-2-benzylbutanoic acid		39.61	6-Acetyl-.beta.-d-mannose
17.97	3,4-Hexanediol, 2,5-dimethyl			
39.61	6-Acetyl-.beta.-d-mannose			
42.19	d-Glycero-d-ido-heptose			

**Fig 9:** Graphical abstract**Fig 10:** Host-Derived Kairomones of Sweet Potato (*Ipomoea batatas*) and Their Attraction to the Sweet Potato Weevil, *Cylas formicarius*

Discussion

Even though 'pests' are also part of nature and is not a constant status as described by (Krishnan *et al.*, 2019) [21], it is essential to check their population explosion through novel methods in commercially cultivated fields. Semiochemicals play an important role in this context either as repellents or as attractants. The sensillae present on the weevil antennae enables the insects for chemical as well as thermal reception (Visser, 1983; Krishnan *et al.*, 2017a) [19, 20, 41]. Technological advances in analytical instrumentation enable us to find out several hundred volatile compounds emanating from fruits and vegetables (Wang and Kays,

2000) [44]. Best yield was observed for many phytochemical extraction studies by employing the method of solvent extraction from dry plant materials. Waksmundzka *et al.*, (2008) [43] and Arceusz *et al.*, (2013) [2] reported a better response by this method from medicinal plants. (Sultana *et al.*, 2009) [36] describes the importance of specific drying of plant materials in extraction technique for the functional activity of phytochemicals. The effective response of sweet potato weevil over the solvent extracts from dried vines in this background was not observed to be effective for the isolation of kairomone chemical species. By this method effective response value of + 3 and -3 was observed to be the

maximum limit at both the end. Solvent extraction method employed in fresh vine cuttings (+ 17 and -4) were almost on par with the more sophisticated cold extraction technique (+ 19 and 0).

Crude extract profile generated through GC-MS for the acetone fraction from the cold extraction technique derives twenty four major compounds of which 2-hydroxy,4-methyl benzaldehyde (Shibata *et al.*, 1998) [34]; 1, 8-di bromo octane (Svirskaya and Leznoff, 1984) [38]; 1-bromo do decane (Rajadhyaksha, 1982) [32]; Phenylacetaldehyde (Wang and Kays, 2002) [45]; Methyl geranate (Wang and Kays, 2002) [45] and Nerolidol iso butyrate (Aldrich *et al.*, 1993) [1] are reported to have kairomonal activity. The compounds identified in this study included heterocyclic compounds, aldehydes, alcohols, ketones, aromatic hydrocarbons, terpenoids, and acids and included 18 compounds not reported previously from sweet potato.

Various saccharides with a caramel like aroma, was demonstrated previously to be an important component in the aroma of baked sweet potatoes (Sun *et al.*, 2019) and the current study confirmed its importance even in fresh vines by the presence of compounds like 6-Acetyl-.beta.-d-mannose; d-Glycero-d-ido-heptose; l-Gala-l-ido-octose. As described by (Tressl *et al.*, 1981, 1989) [30, 31] these simple sugars can act as an efficient attractant when they are heated up in vines with the exposure to direct sunlight and in the presence of amino acids. Phenylacetaldehyde gives a perfume aroma note and is formed *via* Strecker degradation of phenylalanine. It is found widely in many flavors including fruit, chocolate, and honey (Scarpellino and Soukup, 1993) [33]. Phenylacetaldehyde was also observed to be as a major flavouring factor in cooked beef (Gasser and Grosch, 1990; Münch *et al.*, 1997) [11, 25]. Methyl geranate, a relatively little known monoterpene ester in foods, had a pronounced influence on the aroma of baked sweet potato. From our current study, compounds such as 3,4-Hexanediol, 2,5-dimethyl and 9-Acetoxy nonanal were observed to be key components in sweet potato vines.

The exponential growth in the population of a weevil is mainly facilitated by the availability of nutrition for a second reproductive cycle (Krishnan *et al.*, 2016; Krishnan *et al.*, 2017b) [18, 19, 20]. For many species of weevils kairomones act as the response stimuli to direct the pest towards a new host or field. But the characteristic aroma of a number of crops are often derived from only a very small number or even a single volatile compound (Kays, 1991) [17] which might be a small fraction in a cocktail of chemicals, the correlation of volatile constituents with sensory analysis (such as GC-MS-MS-EAG) is essential to determine which odor-active compounds are important (Wang and Kays, 2000) [44]. Study by this technique was an explosion in the semiochemicals study and the legacy was still going on. Even though studies in all class of insect models are available in literature, most of them were from the group Lepidoptera (Hall *et al.*, 1981; Nesbitt *et al.*, 1985; Beever *et al.*, 1986, 1999; Cork *et al.*, 1989) [3, 4, 7, 14], Orthoptera (Carlson *et al.*, 1978; Bursell *et al.*, 1988; Puri *et al.*, 2006) [5, 6, 26, 29] and Coleoptera as described Inn. Mayer and McLaughlin (1991) [22]. All the eight compounds that we observe to be kairomone species where new and were first report, while the compound 6-Acetyl-beta-d-mannose was previously reported in *Aspergillus fumigatus* by Mohammad and Ghaidaa (2016)

The current study clearly indicates the presence of novel phytochemicals present in the sweet potato vines; this may potentially lead for the generation of an attraction lure in the near future. 5 out of the 8 compounds shows sex neutral attraction in the GC-MS-MS-EAG study. Specific purification and field standardisation studies on these five compounds will replace the existing sex pheromone attractants of *C. formicarius*.

Conflict of interest

Authors declare no conflict of interest as defined by this publishing group, or other interests that might be perceived to influence the results and/or discussions reported in this article.

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