



Original Research Article

GC - MS Analysis of *Bruchus pisorum* Extract to Analyze the Presence of Semichemical Components

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ABSTRACT

Keywords

Bruchus pisorum,
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Semichemical,
Ethanollic
extract

Bruchus pisorum, a cosmopolitan pest of field peas, was first recorded in South Africa in 1964. Although this pest is called pea weevil, it is not a true weevil like the granary and rice weevils. Unlike them, pea weevil cannot reproduce in stored grain. Infestations of pea weevil can only occur if eggs are laid onto green pea pods in the field. In the present investigations CG-MS analysis was carried out to quantify the different compounds present in the *Bruchus pisorum*. The result shows 19 compounds are present in the extract of *Bruchus pisorum*. Out of 19 compounds Isopropyl tetradecanoate used for head lice treatment and 2, 5-Hexanedione, 3, 4- dimethyl is found to be neurotoxic.

Introduction

Insects and pests are a major constraint to increase global production of food. There are an estimated 67000 pest species that damage agricultural crops. *Bruchus pisorum* is a pest belongs to Family Bruchidae(1,2). This pest is responsible for damaging the pea. It was first recorded in South Africa in 1964 (Greg Baker, Senior Entomologist). The adult is a chunky beetle about 5 mm long, generally brownish flecked with white, black and grey patches. The tip of the abdomen extends beyond the wing covers and is white marked with two black oval spots(3).The egg is yellow, cigar-shaped and measures 1.5 mm by 0.6 mm. The larva is a legless, curled, cream grub which grows to about 5 mm long.

Heavily infected pea crops may have up to 15 to 20 per cent of the pea seeds attacked by pea weevil larvae. Infested seeds can lose up to 25 percent of their weight from larval feeding, and are prone to shattering when harvested, so that the total yield of a heavily infested crop may be reduced by 5 to 10 percent. The species causes damage to pea plants (4,5). These grains are not suitable for sowing.

Intensive colonization of field edges by beetles is observed during the period of mass oviposition. Problems have been encountered in obtaining resistance sources against a particular pest and emergence of resistance. These problems can now be

overcome by using modern cellular and molecular approaches to develop resistance across conventional barrier to reproduction into other related or even unrelated crop species (6,7 & 8). Control measures include early harvesting of grain, stubbling, under-winter plowing, fumigation of grain in granaries, and insecticide treatments in fields during the period of budding and blossoming (13, 14 & 15).

Materials and Methods

Sample collection

Bruchus pisorum was collected from Raipur (Chhattisgarh), India.

Extraction preparation

Ethanol extract was prepared by crushing the *Bruchus pisorum* with 10ml of ethanol with the help of mortar and pestle. Then it is filtered through Whattmann no. 1 filter paper. Then the filtrate was collected and centrifuged. The centrifuged sample was allowed for GC-MS analysis(6).

GC-MS (Gas Chromatography-Mass Spectrometry)

GC-MS is a technique that can be used to separate volatile organic compounds (VOCs). The Gas Chromatography- Mass Spectrometry (GC-MS) instrument separates chemical mixtures (the GC component) and identifies the components at a molecular level (the MS component). It is one of the most accurate tools for analyzing environmental samples. The GC works on the principle that a mixture will separate into individual substances when heated. The heated gases are carried through a column with an inert gas (such as helium). As the separated substances emerge from the column opening, they flow into the MS.

Mass Spectrometry identifies compounds by the mass of the analyte molecule. A library of known mass spectra, covering several thousand compounds, is stored on a computer. Mass Spectrometry is considered the only definitive analytical detector.

Conditions

Column Oven Temperature: 70°C
Injector Temperature : 200°C
Injection Mode : Split
Split Ratio : 40
Flow control mode: Linear velocity
Column Flow : 1.51ml/min
Carrier Gas : Helium 99.9995% purity

Column oven temperature program

Rate	Temperature (°C)	Hold Time (min)
-	70	2
10	300	10 (35.0MIN)

Column: VF-5ms
Length: 30.0m; Diameter: 0.25mm
Film Thickness: 0.25um

Results and Discussion

GC-MS analysis

The extract prepared was analyzed for the presence of semiochemical components using GC-MS. The result shows that 19 different compounds were present in it (Fig. 3).

Isopropyl tetradecanoate

Head lice infestation is common, and mainly affects children of primary school age. Isopropyl tetradecanoate 50% in cyclomethicone solution is a new fluid treatment with a physical mode of action that uses a 10-minutes contact time. This

compound it beneficial for head lice treatment (Drug and therapeutics bulletin, May 2007 Pub Med).

2, 5-Hexanedione, 3, 4-dimethyl

2, 5-Hexanedione, 3, 4-dimethyl was characterised to be a neurotoxin. It reduces the rate of transport of the neurofilament protein (12).

Bruchus pisorum is a pea pest and a major constraint to increase global production of

food. This pest is responsible for damaging the pea. In the present investigations CG-MS analysis shows 19 compounds are present in the extract of *Bruchus pisorum*. Out of 19 compounds as per previous studies found Isopropyl tetradecanoate used for head lice treatment and 2, 5-Hexanedione, 3, 4- dimethyl is found to be neurotoxic. It reduces the rate of transport of the neurofilament protein(9, 10, 11 &12).

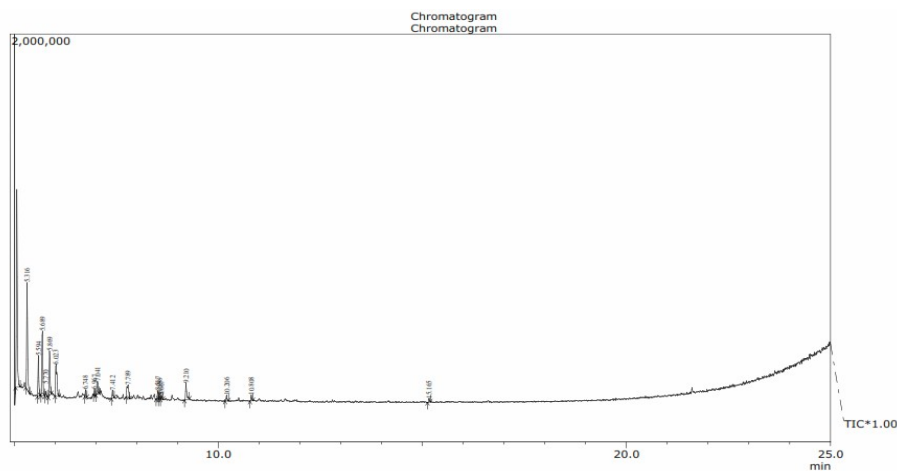
Fig.1 seed infected by *Bruchus pisorum*



Fig.2 Emerged adult, on infected seeds



Fig.3 GC-MS analysis of *Bruchus pisorum* extract



Peak Report TIC

PEAK#	R.TIME	AREA	AREA%	NAME
1	5.064	1669571	31.57	2,2,4-Trimethylpentanol
2	5.316	885744	16.75	1-Isopropyl-2-methylpropyl acetate
3	5.594	317855	6.01	tert-Butyl glycidyl ether
4	5.689	597018	11.29	3,4-Dimethyl-3,4-hexanediol
5	5.770	47151	0.89	3-(2-METHYL-1,3-DIOXOLAN-2-YL)-TETRAHYDRO-2-FURANONE
6	5.869	355088	6.71	Isopropenyl acetate
7	6.023	442487	8.37	3,4,5-Trimethyl-4-heptanol
8	6.748	49546	0.94	Diethylene glycol diacetate
9	6.967	40718	0.77	3-METHYLHEPTAN-2-ONE
10	7.041	142076	2.69	3-Hydroxy-2,2,4-trimethylpentanenitrile
11	7.412	69540	1.31	Pentanal, 3-(acetyloxy)-2,2,4-trimethyl-
12	7.789	215077	4.07	4-Dodecanol
13	8.517	64001	1.21	1-[2-(2-Methyl-5-oxo-4-propyl-tetrahydro-furan-2-yl)-2-oxo-ethyl]-1,2-dihydro-pyridazine-3,6-dione
14	8.558	65500	1.24	3-Methyl-2-heptanone
15	8.607	32848	0.62	2-HEXANONE, 3,4-DIMETHYL-
16	9.210	164538	3.11	2,5-Hexanedione, 3,4-dimethyl-
17	10.206	46111	0.87	(3E)-5,5,7-TRIMETHYL-3-OCTENE-2,6-DIONE
18	10.808	48417	0.92	4,5-DIMETHYL-4-IMIDAZOLIN-2-ONE
19	15.165	35532	0.67	ISOPROPYL TETRADECANOATE
		5288818	100.00	

Fig.4 Isopropyl tetradecanoate

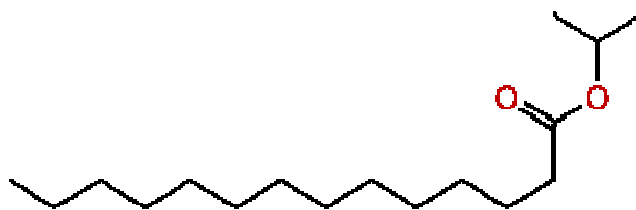
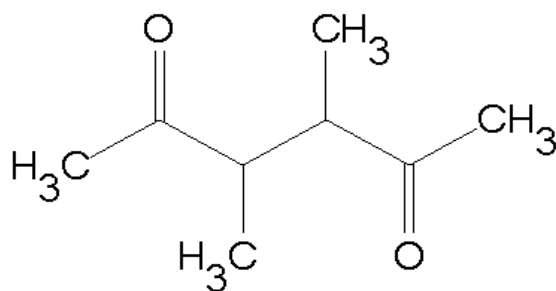


Fig.5 2, 5-Hexanedione, 3, 4-dimethyl



Abbreviations: GC-MS - Gas Chromatography- Mass Spectrometry; VOCs - Volatile Organic Compound

References

1. Roger L. Morton, Hart E. Schroeder, Kaye S. Bateman, Maarten J. Chrispeels, Eric Armstrong, and Thomas J. V. Higgins, (2000)
2. H.E.Schroeder *et al.*, Bean [alpha]-Amylase Inhibitor Confers Resistance to the Pea Weevil (*Bruchus pisorum*) in Transgenic Peas (*Pisum sativum* L.). *Plant Physiol* V.107 (4); 1995 April.
3. Brudnaya A.A. 1940. Natural enemies of pea weevil (*Bruchus pisorum* L.). In: Vavilov N.I., ed. Reports of All-Union Agricultural Academy, N 12. Moscow: Sel'khozgiz. P. 6-10 (in Russian).
4. Burov V.N. 1967. Significance of population density for *Bruchus pisorum* L. population dynamics (Coleoptera, Bruchidae). *Zoologicheskii zhurnal* 46(9): 1357-61.
5. Kadamshoev M. 1985. To biology and damage of pea weevil (*Bruchus pisorum* L., Coleoptera, Bruchidae) in conditions of West Pamir. *Izvestiya Akademii Nauk Tadzhikskoi SSR* 2: 73- 6
6. Vasil'ev I.V. 1939. Origin and world distribution of pea weevil (*Bruchus pisorum* L.). *Vestnik zashchity rastenii* 1. P. 44-5
7. H.C.Sharma *et al.*, Insect pest management in food legumes: The future strategies. *Food Legumes for Nutritional Security and Sustainable Agriculture*, Vol. 1: 522-544. 2008.
8. Annis, B. and L.E. O'Keefe. 1987. Influence of pea genotype on parasitization of the pea weevil, *Bruchus pisorum* (Coleoptera: Bruchidae) by *Eupteromalus Zeguminis* (Hymenoptera: Pteromalidae). *Environmental Entomology*, 16: 653-655
9. Baker, G.H. 1990a. Natural enemies of pea weevil, *Bruchus pisorum* (Bruchidae), worthy of consideration as biological control agents. p. 80-83. In: A.M. Smith (ed.) *Workshop Proceedings: National Pea Weevil Workshop*. Victoria Department of Agriculture and Rural Affairs, Melbourne, Australia.
10. Horne, J. and P. Bailey. 1991. *Bruchus pisorum* L. (Coleoptera: *Bruchidae*) control by knockdown pyrethroid in field peas. *Crop Protection*, 10: 53-56.
11. Morton, H.E. Schroeder, K.S. Bateman, M.J. Chrispeels, E. Armstrong and T.J.v. Higgins. 2000. Bean u- amylase inhibitor r in transgenic peas (*Pisum sativum*) provides complete protection from pea weevil (*Bruchus pisorum*) under field conditions. *National Academy of Sciences, USA*, 97
12. John W. Griffin *et al.*, 2, 5-Hexanedione, 3, 4-dimethyl impairs the axonal transport of neurofilament proteins. *The journal of Neuroscience* vol. 4. No. 6. June 1984.
13. Effect of pollen source on oogenesis in the pea weevil, *Bruchus pisorum* L. (Coleoptera: Bruchidae). Annis, B.; O'Keefe, *Journal Protection Ecology* 1984 Vol. 6 No. 4 pp. 257-266.
14. Pea weevil, *Bruchus pisorum* L. (Coleoptera: Bruchidae), resistance in *Pisum sativum*, *Pisum fulvum* interspecific crosses. S. L. Clement, K. E. McPhee, L. R. Elbersen and M. A. Evans. *Plant Breeding* Volume 128, Issue 5, pages 478-485, October 2009
15. Response of *Np* mutant of pea (*Pisum sativum* L.) to pea weevil (*Bruchus pisorum* L.) oviposition and extracts. Robert P. Doss, William M. Proebsting, Sandra W. Potter, Stephen L. Clement. *Journal of Chemical Ecology*, January 1995, Volume 21, Issue 1, pp 97-106.