Print ISSN : 0972-8813
e-ISSN : 2582-2780

[Vol. 19(1), January-April, 2021]

Pantnagar Journal of Research

(Formerly International Journal of Basic and Applied Agricultural Research ISSN : 2349-8765)

G.B. Pant University of Agriculture & Technology, Pantnagar

PANTNAGAR JOURNAL OF RESEARCH

Vol. 19(1) January-April, 2021

CONTENTS

Bio-efficacy of *Ageratum houstonianum* Mill. (Asteraceae) essential oil against five major insect pests of stored cereals and pulses

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ABSTRACT: Fumigant toxicity of essential oil of *Ageratum houstonianum* Mill. (Asteraceae) was studied against five major insect pests of stored grain, namely, Rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), Lesser Grain Borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae), Red Flour Beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and Pulse Beetle, *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae). Essential oil of *A. houstonianum* was extracted in Clevenger apparatus by hydro-distillation technique. GCMS analysis of essential oil revealed that ageratochromene (precocene II) (32.02), trans β-caryophyllene (23.06), precocene I (8.5), E-β-farnesene (6.49), β -cubebene (4.33), sesquisabinene (4.04), D-germacrene (3.05), benzyl nitrile (2.11), α-humulene (1.36), camphene (1.24), amorphene-gamma (1.22), α-farnesene (1.2), δ-cadinene (1.13), β-geraniolene (1.1), and α-phellandrene (0.27) are major component of it. Fumigation toxicity of essential oil was studied at 0.2, 0.1 0.05, 0.025 % V/W in airtight plastic vials. Ten adults of test insect were released in treated food and observation was recorded on emergence of F_1 generation to calculate per cent inhibition as compared to untreated control. Fumigation of grain resulted in 91.42 per cent inhibition of F_1 progeny of *S. oryzae* at 0.20 per cent while 100 per cent inhibition was recorded in case of *R. dominica* and *C. chinensis* at this level. As compared to these insects, the essential oil was less effective against *T. castaneum* and *S. cerealella,* respectively, against whom only 78.50 and 55.78 per cent inhibition was observed at 0.20 per cent. In almost all cases, the fumigant toxicity increased with increasing concentrations. The results indicated that essential oil of *A. houstonianum* may be explored for eco-friendly management of *R. dominica, S. oryzae* in cereals and *C. chinensis* in pulses.

Key words: *Ageratum houstonianum, Callosobruchus chinensis,* fumigant toxicity, *Rhyzopertha dominica, Sitotroga cerealella*, *Sitophilus oryzae, Tribolium castaneum*

Essential oils present in the plants of several families are known to exert harmful effect on the survival, growth and development of many insects injurious to crop plants and stored grain. They are complex mixture of hydrocarbons and oxygenated hydrocarbons and mainly consist of monoterpenes and sesquiterpenes. Investigations made on some essential oils have revealed that it may disrupt the behaviour, physiology, vigour, longevity and fecundity of insect in various ways and even prove toxic to different developmental stages of many insects (Don-Pedro, 1996; Koul and Dhaliwal, 2001; Clemente *et al*., 2003). Some oils have also been found to work as repellent, anti-feedant, ovicidal, or oviposition inhibitors in insect pest (Pascual-villalobos and Ballesta-Acosta, 2003). Although, pest control properties of plants are known to human being from pre-historic period, scientific study on it started in the beginning of twentieth century (Jilani, 1984). However, not much attention on this aspect could be paid till 1960 after which several attempts have been made to find out the plants with insect control properties. The advancement made in this field in past six decades have proved beyond doubt that essential oils present in plants have adequate potential to protect stored grain from insect infestation if comprehensive studies are taken to explore the pest control properties in these vast natural resources.

Essential oil from more than seventy five plant species belonging to different families, such as Anacardiaceae, Apiaceae (Umbeliferae), Araceae, Asteraceae (Compositae), Brassicaceae (Cruciferae) Chenopodiaceae, Cupressaceae, Graminaceae, Lamiaceae (Labiatae), Lauraceae, Liliaceae, Myrtaceae, Pinaceae, Rutaceae and Zingiberaceae have been studied for fumigant toxicity against insect pests of storage grain (Grainge and Ahmed, 1988; Rajendran and Sriranjinia, 2008; Geetanjly *et al.,* 2016; Gangwar and Tiwari, 2017; Kumar and Tiwari, 2017a; 2017b; 2018 a; 2018b; Joshi and Tiwari, 2019; Kumar and Dubey, 2020). Considering the advantages which essential oils of plant origin offer in being easily available cheap, eco-friendly and biodegradable (Jacobson, 1983), it is imperative to identify more and more plants suppressing 90-100 per cent survival, feeding and breeding of different stored grain insects. Being one of the highly diversified regions of flora, our country may play leading role in this direction. As the fumigants of botanical origin are much more useful as compared to chemical fumigants there is a necessity to evaluate as much essential oils as possible as it may lead to the development of a safe, cost effective and environmental friendly method of pest control in stored grain.

Ageratum conyzoides L. and *Ageratum houstonianum* Mill. belonging to family Asteraceae are two very important weed which are widely distributed in several countries. Some experiments have been conducted on the fumigant toxicity of *A. conyzoides* against *R. dominica*, *S. oryzae* and *T. castaneum* (Nenaah, 2014; Singh *et al.,* 2014) and *C. maculatus* (Gbolade *et al.,* 1999), however, we do not have such information about *A. houstonianum.* Since this species is also found abundantly in many countries, present study was conducted with an objective to investigate the fumigant toxicity of its essential oil against five major insect pests of stored grain so that it could be utilized for eco-friendly management of these insect pests.

MATERIALS AND METHODS

Preparation of culture

Pure culture of each test insect was developed separately in the plastic jars of about 1 liter capacity at 27±1 ºC temperature and 70±5 per cent relative humidity. For proper aeration in the jar, its cap was provided with 1.8 cm diameter hole which was covered with 30 mesh copper wire net. The culture of *R. dominica* and S. *oryzae* was developed on wheat variety PBW-502 maintained at 13.5 per cent moisture content while *S. cerealella* was reared on the paddy grain on the same moisture. The culture of *T. castaneum* was prepared on wheat flour fortified with yeast powder at the rate of 5 per cent while chickpea seed of variety Pusa-362 was used to culture *C. chinensis.* The flour for rearing *T. castaneum* was prepared after maintaining the moisture content of wheat variety PBW-502 at 13.5 per cent. Before maintaining the moisture content, the grain was disinfested in the oven at 60 ºC for 12 hrs after which its moisture content was measured by Universal Moisture Meter. To raise the moisture content of grain to 13.5 per cent, measured quantity of water calculated as per formula given by Pixton (1967) was sprayed on the grain by hand atomizer after spreading it on polythene sheet. The grain was turned after each spraying for uniform coating of water on grain surface. After mixing the water in the grain it was stored in airtight container for a period of one week so that moisture could equilibrate in all the grain. After moisture equilibration, 500g grain was filled in plastic jar in which 100 adults of same age were released for feeding and breeding. All the released adults were removed from the jar after one week. First generation adults emerged within one week were used for experimental purpose.

Preparation of experimental medium

Whole grain of wheat variety PBW-502 was used to conduct experiment on *R. dominica, S. oryzae* and S. *cerealella* while it was broken to conduct experiment on *T. castaneum*. Whole seed of chickpea variety Pusa-362 was used for experiment on *C. chinensis.* The moisture content of experimental medium was maintained as per details given above.

Extraction of oil

Whole plants of *A. houstonianum* were collected from N.E. Borlaug Crop Research Center Pantnagar which was washed thoroughly in sufficient water. After removing the water by multiple shaking they were semi-dried under shade before steam distillation. The distillation process was done in Clevenger Apparatus (Cat. No. 475/4, Jain Scientific Glass works). Anhydrous sodium sulphate was used to remove any trace of moisture and stored in air tight container in a refrigerator at 4^0C until used for experiment.

GCMS analysis of essential oil

GC-MS analysis of essential oils was performed in Advanced Instrumental Research Facility (AIRF), Jawahar Lal Nehru University (JNU) New Delhi, using following programme: Sampler: high plunger speed (Suction), 0.2 sec viscosity comp. Time, high plunger speed (injection), high syringe insertion speed, Normal injection mode. GC: 50.0^0C column oven temp, 260.0^0C injection temp, split injection mode, 1.00 min sampling time, Helium as carrier gas, prim. Press 500-900, linear velocity flow control mode, 69 kPa pressures, 125.2 ml/min total flow, 1.2 ml/min column flow, 39.3 cm/sec linear velocity. 3.0 ml/min purge flow, 100.0 split ratios. Column: name Rtx-5ms, 0.25um thickness, 30.0m length, 0.25mm diameter, GC program time 70.08 min. MS: 220^0C ion source temp, 270^0C interface temp, 3.5min solvent cut time, and Start time 4min, End time 70.08 min. The data obtained after GCMS analysis was compared with the library (FFNSC 2.LIB, WILEY8.LIB, NIST14.LIB, NIST14s.LIB and SZTERP.LIB) available there in the system to identify the essential oil components.

Bio-efficacy against insect pests

The experiment was conducted under controlled conditions at 27 ± 1^0C temperature and 70 ± 5 per cent relative humidity. Airtight plastic vials (115 ml) were used for fumigation of grain contained in it. The bioassay on *R. dominica, S. oryzae* and *S. cerealella* was conducted on wheat grain of variety PBW-502 (13.5 per cent moisture) while broken wheat grain of variety PBW-502 (13.5 per cent moisture) was used for experiment on *T. castaneum.* Chickpea seed of variety Pusa-362 (13.5 per cent moisture) was used for bioassay on *C. chinensis.* Each treatment was replicated three times and untreated grain was used as control. Fifty gram food (moisture content 13.5 per cent) was filled in plastic vial and ten 0-7 days' old adults of *R. dominica, S. oryzae* or *T. castaneum* were released in respective vial. In case of *C. chinensis* only 24 h old adults were released in plastic vial. After 24 h of releasing the insects, measured quantity of essential oil (0.20, 0.10, 0.05 and 0.025 % V/W) was poured on the absorbing paper mat, which was then placed inside the grain filled in vial. After closing the screw cap of vial tightly it was sealed by paraffin film to make the vial completely airtight. In case of *S. cerealella,* oil treated mat were inserted first in the grain filled in the vial after which ten 24 h old adults insects were released in it. Insects were then allowed to feed and breed for one month. Observation was recorded on F_1 progeny by counting adults emerged in each vial after one month and per cent inhibition was calculated using the following formulae:

Per cent Inhibition = [(AC-AT)/(AC)] x 100
Where:
AC: F_1 adult count in control
AT: F_1 adult count in Treatment (Tapondjou *et al.,* 2002).

Statistical analysis

Completely Randomized Design was used for data analysis after suitable log transformation by using SPSS. Data is also depicted through graph for better understanding.

RESULTS AND DISCUSSION

GCMS analysis

GCMS analysis revealed that ageratochromene (precocene II) (32.02) is the major component of *A. houstonianum* followed by trans-β-caryophyllene (23.06), precocene I (8.5), (E) β- farnesene (6.49), β-cubebene (4.33), sesquisabinene (4.04), germacrene D (3.05), benzyl nitrile (2.11), α-humulene (1.36), camphene (1.24), amorphene-gamma (1.22), α-farnesene (1.2), δ-cadinene (1.13), β-geraniolene (1.1), and α-phellandrene (0.27) (Figure 1 and Table 1). However, essential oil analysed by Chandra *et al.* (1996) indicated precocene II, precocene I and β-caryophyllene in the ratio of 43.99%, 23.34% and 9.18%, respectively. On the other hand precocene-II (52.64%), precocene-I (22.45%) and β-caryophyllene (9.66%) have been reported to be major components of essential oil of *A. houstonianum* **(**Kurade *et al.,* 2010). The oil analysed by Lu *et al.* (2014) contained precocene II, precocene I and β-caryophyllene in the ratio of 62.68%, 13.21% and 7.92%, respectively. A different composition have been reported in the essential oil of *A. conyzoides* the major constituents of which are 6-demethoxyageratochromene (precocene I) (80.29%) and beta β-caryophyllene (7.04 %) (Mensah *et al.,* 1993). All these studies indicate that fumigant toxicity of essential oil of *A. houstonianum* may be due to precocene II, precocene I and β-caryophyllene which are found in maximum amount. It has been reported that difference in the chemical composition of plant essential oils might arise from climatic, seasonal and geographical differences in addition to chemo type and genetic variations, harvesting time, nutritional status of the plant and the method of extraction (Perry *et al.,* 1999; Rahimmalek *et al.,* 2009 and Nenaah E, 2014).

Table 1: Major components in *Ageratum houstonianum* essential oil

S. No.	Retention Time	Area	Area (%)	Name
1	7.054	13643859	1.24	Camphene
2	8.595	12095946	1.10	B-Geraniolene
3	9.189	2943369	0.27	α-Phellandrene
4	15.198	23165866	2.11	Benzyl nitrile
5	25.793	47671462	4.33	β-Cubebene
6	27.207	253574199	23.06	Trans β-Caryophyllene
7	28.483	14950871	1.36	α-Humulene
8	28.695	71428246	6.49	E β-Farnesene
9	29.15	93463799	8.50	Precocene I
10	29.625	33570780	3.05	Germacrene D
11	30.015	13378359	1.22	γ-Amorphene
12	30.755	13205645	1.20	α-Farnesene
13	31.141	12419507	1.13	δ-Cadinene
14	31.438	44377671	4.04	Sesquisabinene
15	36.839	352103867	32.02	Ageratochromene (Precocene II)
	Total	1001993446	91.12	

*C. c, *Callosobruchus chinensis*; R. d, *Rhyzopertha dominic*a; S. o, *Sitophilus oryzae*; T. c, *Tribolium castaneum*; S. c, *Sitotroga cerealella*.

Bio-efficacy against insect pests

Remarkable difference in the bio-efficacy of essential oil

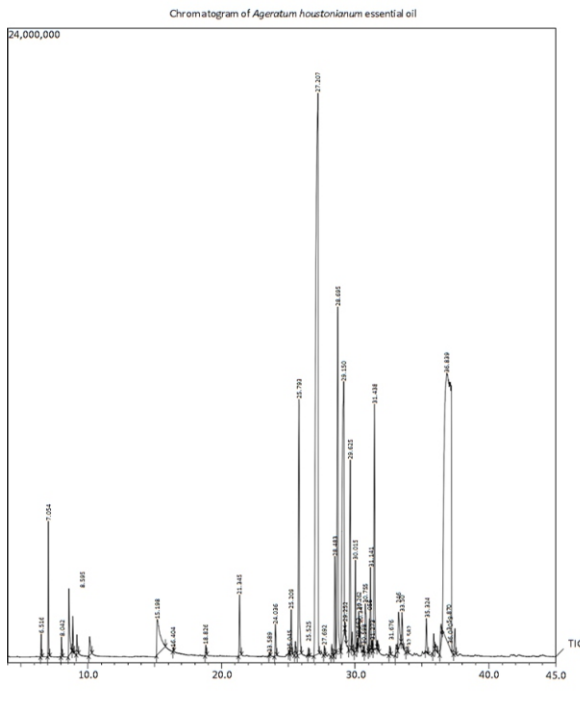

Figure 1: Chromatogram of *A. houstonianum* after GCMS analysis

Table 2: Number of adult emerged in the grain treated with different concentration of essential oil of *Ageratum houstonianum*

Concentration (% V/W)	*Sitophilus oryzae*		*Rhyzopertha dominica*		*Sitotroga cerealella*		*Callosobruchus chinensis*		*Tribolium castaneum*	
	Number of adult emerged	Percent Inhibition	Number of adult emerged	Percent Inhibition	Number of adult	Percent Inhibition	Number of adult emerged	Percent Inhibition	Number of adult emerged	Percent Inhibition
0.200	24.33(1.33)	91.42	0.00(0.00)	100.00	23.67(1.35)	55.78	0.00(0.00)	100.00	15.33(1.16)	78.50
0.100	47.67(1.67)	83.20	2.33(0.42)	96.65	30.33(1.43)	46.55	0.00(0.00)	100.00	25.00(1.39)	64.95
0.050	50.67(1.70)	82.14	6.67(0.88)	90.43	34.67(1.54)	45.37	0.00(0.00)	100.00	29.33(1.47)	58.88
0.025	62.33(1.79)	78.03	12.00(1.11)	82.78	49.00(1.69)	40.75	0.00(0.00)	100.00	31.67(1.50)	55.61
Control	283(2.44)		69.67(1.85)		96.33(1.98)		52.67(1.69)		71.33(1.85)	
SEM(±)	(0.06)		(0.12)		(0.11)		(0.06)		(0.073)	
CD(0.05)	(0.25)		(0.33)		(0.30)		(0.17)		(0.199)	

SEM(±)= Satandard Error of Mean; CD= Critical Difference at 5%. Data in parenthesis indicate log transformed value

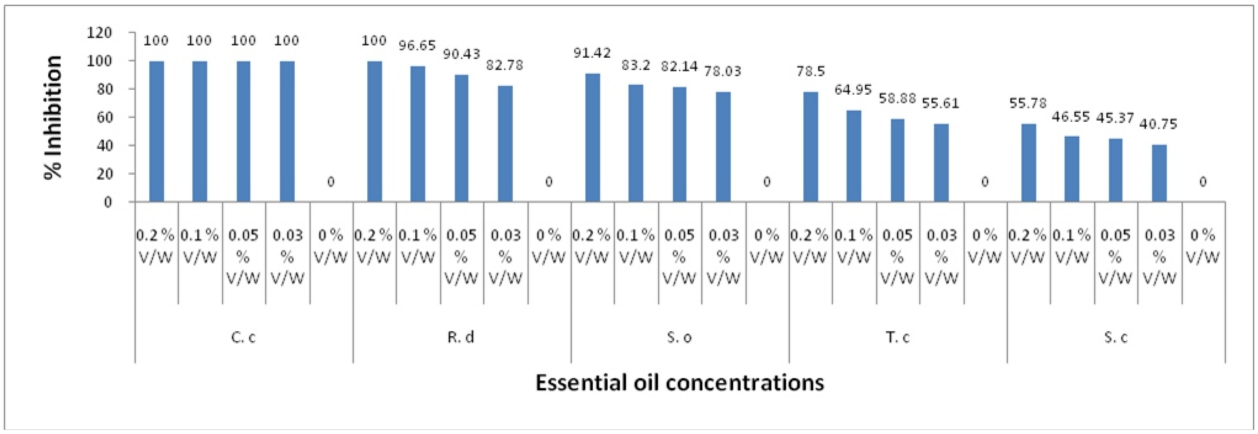

Figure 2: Per cent inhibition shown by essential oil against five storage pest species

of *A. houstonianum* was noticed against different insect species. It was found to be most effective against *C. Chinensis,* the reproduction of which was inhibited completely even at lowest concentration of 0.025 per cent. No F_1 adults emerged from the seed fumigated by this essential oil at 0.025, 0.05, 0.10 and 0.20 per cent (V/W) while 52.7 adults emerged in untreated control (Table 2; Figure 2). The efficacy of this insect decreased slightly in case of *R. dominica* in case of which 82.78, 90.42, 96.65 and 100 per cent inhibition of F_1 progeny was achieved at 0.025, 0.05, 0.10 and 0.20 per cent (V/W), respectively. Further reduction in the efficacy of essential oil was noticed against *S. oryzae* which succeeded to produce 62.33, 50.67, 47.67 and 24.33 F_1 adults in the grain treated at 0.025, 0.05, 0.10 and 0.20 per cent (V/W), respectively, while 283 adults emerged in untreated control. Treatment of grain at 0.025, 0.05, 0.10 and 0.20 per essential oil of *A. houstonianum* resulted in 78.03, 82.14, 83.20 and 91.42 per cent suppression of F_1 progeny of *S. oryzae,* respectively. The bio-efficacy of essential oil reduced further in case of *T. castaneum* as treatment of grain at 0.025, 0.05, 0.10 and 0.20 per cent resulted in 55.61, 58.88, 64.95 and 78.50 per cent inhibition of F_1 progeny, respectively. Lowest efficacy of this oil was observed against *S. cerealella* in case of which only 40.75, 45.37, 46.55 and 55.78 per cent inhibition was recorded at 0.025, 0.05, 0.10 and 0.20 per cent, respectively. The results indicated that essential oil of *A. houstonianum* is effective against test insects in the sequence of *C. chinensis*> *R. dominica*> *S. oryzae*> *T. castaneum*> *S. cerealella*.

A comparison of bio-efficacy of *A. houstonianum* with *A. conyzoides* indicated that both the oils showed more or less similar trend against *R. dominica*, *S. oryzae* and T. *Castaneum.* Application of essential oil of *A. conyzoides* at 60 µl/L air resulted in 100, 89.8 and 62 per cent reduction in F1 progeny of *R. dominica, S. oryzae* and *T.castaneum*, respectively (Nenaah, 2014). It is presumed that Precocen I and Precocen II which are the major components in the essential oil of *A. conyzoides* and *A. houstonianum* are responsible for adverse effect of different organism,

CONCLUSION

It may be concluded that essential oil of *A. houstonianum* is most effective against *C. chinensis* even at 0.025

percent at which it may be used for eco-friendly management of this insect. Its effect is also appreciable at 0.20 per cent against *R. dominica* and *S. oryzae.*

ACKNOWLEDGEMENTS

Authors are thankful to Dr. Ajay Kumar (System Analyst), Advanced Instrumental Research Facility (AIRF), Jawahar Lal Nehru University (JNU) New Delhi, for analysis of essential oils through GCMS.

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Received: April 4, 2021
Accepted: April 29, 2021