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Aloe vera (L.) Burm. f. : A highly useful Indian traditional plant for the management of maize storage pest, *Sitophilus oryzae* L. (Coleoptera: Curculionidae)

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ABSTRACT

Aloe vera (L.) Burm. f. is an important Indian traditional plant possessing wide range of biological activities. The present study is aimed to determine the repellent and contact toxicity of methanolic extracts of gel, root and leaf peel parts of fifteen *A. vera* accessions collected from 15 locations in India against maize storage pest, *Sitophilus oryzae* (L.). Petri dish choice bioassay and filter paper impregnation methods were used to determine repellent and contact toxicity respectively. Analysis of the results reveals that aloin A, the major metabolite of *A. vera*, at 0.02% w/v concentration possessed strong repellent activity (85.2 %) than sample AV 11a (gel of *A. vera*) at 5% w/v (82.7%) after 24-h of exposure. Interestingly, the percent repellency increases up to 5-h in all extracts screened. Aloin A at 0.0024 mg/cm^2 showed 58.0% mortality against *S. oryzae* followed by sample AV 13b (leaf peel) at 0.6 mg/cm^2 (56.0%) after 14 days of treatment. The content of Aloin A present in the six *A. vera* extracts, exhibiting highest toxicity and repellency, was determined by Ultra Performance Liquid Chromatography. The percent repellency and toxicity of *A. vera* vary with geographical location, plant parts, major metabolite concentration and exposure time. The potentiality of these plant extracts could be useful towards the development of effective bio repellent to *S. oryzae* in stored maize.

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INTRODUCTION

Aloe vera (Linn.) Burm. f. (Liliaceae) is a highly potent Indian traditional plant, found with many health benefits such as wound healing (first and second degree burns), improvement of digestion and blood glucose levels; reduces fatigue and fungal infection (Rajeshwari *et al*., 2012). Phytochemical constituents of *A. vera* include high content of phenolic compounds, glycosides (aloins), 1, 8 dihydroxy anthraquinones (aloe emodin), β-1,4 acetylated mannan, mannose phosphate, and alprogen gluco protein (Sharma, 2014). Aloin A (hydroxy anthrone glycoside) is the major constituent of *A. vera* (Yadav, 2015) with smaller amounts of its C-10 epimer, aloin B. Aloin A has numerous biological activities such as antimicrobial, antifungal (Renuka *et al*., 2012); antibacterial, anti oxidant, cytotoxic drug against ovarian tumour cell lines. The leaf exudate and gel of *A. vera* also possess antifungal, antibacterial, anticancer, antioxidant, cryoprotective, immune modulatory (Patel *et al*., 2012) and insecticidal activities (Morsy *et al*., 2000). The phyto sterols, lophenol, 24-methyl-lophenol, 24-ethyl-lophenol, cycloartanol, and 24-methylene-cycloartanol were isolated from the gel of *A. vera*, some of those metabolites have long-term blood glucose level control effect (Tanaka *et al*., 2006).

The utilization of naturally available plants as effective biopesticides has been in long use for the control of storage pests in India (Rajasekhar *et al*., 2012). Plant extracts or

fractions or isolated pure compounds have been recognized as important natural agents to control some of the storage pests (Usha Rani *et al*., 2014; Ribeiro *et al*., 2014). Rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) cause grain damage of 53.30% resulted in weight loss of 14% in stored maize (LakshmiSoujanya *et al*., 2013). It is the most destructive insect pest of maize particularly during storage. Synthetic insecticides have been widely in use for the control of *S. oryzae*, which ultimately lead to resistance to phosphine and other chemicals (Daglish *et al*., 2014). Application of insecticides also results in the accumulation of residues on grains leading to health and environmental hazards (Sharma and Meshram, 2006). According to Montreal International agreement, the use of fumigants such as methyl bromide has been banned due its ozone depletion effect (Singh and Sharma, 2015). Therefore, botanical insecticides are highly desirable for the integrated management of (IPM) storage pests and can be used as alternative sources to conventional insecticides (Tapondzou *et al*., 2005; Wang *et al*., 2006; Dubey *et al*., 2008). It has been reported in literature that some plant species found to be repellent and toxic to *S. oryzae* (Talukder and Howse, 1994; Paranagama *et al*., 2004; Samir *et al*., 2009; UshaRani, 2012). Some reports are also available on the biological activities of plant species belonging to Liliaceae against storage pests (Denloye and Makanjuola, 1997; Huang *et al*., 2000; Denloye, 2010; Nwachukwu and Asawalam, 2014). Farmers of East Africa use leaves of *Aloe spp* to protect the grain against storage pests. However, limited information is available on the use of *Aloe vera* for stored product protection. Therefore, we focused on various parts of *Aloe vera* accessions collected from different localities in Andhra Pradesh, Telangana and Rajasthan states of India and determined their repellent and contact toxicity against *S. oryzae*.

MATERIALS AND METHODS

Plant material

A total of fifteen different *A. vera* accessions were collected from 15 localities of Andhra Pradesh, Telangana and Rajasthan states of India. The three plant parts such as gel (a), leaf peel (b) and root (c) were separated and coded as follows (Refer Table 1.): AV1 - Adilabad, (19° 40' 0" N,78° 32' 0" E) Telangana; AV 2 - Kadiri (14° 07' N, 78° 14' E), Andhra Pradesh; AV 3 - Tissue culture sample of Central Institute of Medicinal and Aromatic Plants (CIMAP), Hyderabad (17.3850° N, 78.4867° E), Telangana; AV 4 - Mother plant of CIMAP, Hyderabad, Telangana; AV 5 – Prakasham (15°20′N, 79°33′E), Andhra Pradesh; AV 6 - YSR Horticultural University, Hyderabad, Telangana; AV 7 - Khammam (17.2473° N, 80.1514° E) , Telangana; AV8 - National Bureau of Plant Genetic Resources (NBPGR), Jodhpur (26.2389° N, 73.0243° E), Rajasthan; AV 9 – Tirupathi (*13.629065, 79.424446)*, Andhra Pradesh; AV 10 - Kurnool (15.8281° N, 78.0373° E), Andhra Pradesh; AV 11 a, b, c – Karimnagar (18.4386° N, 79.1288° E) Telangana; AV 12 - NBPGR, Hyderabad, Telangana; AV 13 – Medak (18° 03' N, 78° 18' E) Telangana; AV 14 – Rajahmundry (17.0005° N, 81.8040° E), Andhra Pradesh and AV 15 - Guntur, (16.3067° N, 80.4365° E) Andhra Pradesh.

Extract preparation

Different parts of *A. vera* such as leaf peel, gel and root were separated and shade dried for one week at room temperature (26-28^{o}C). The dried materials were finely powdered using mixer grinder. The root and leaf peel (each 10 g) were extracted individually with methanol (200 mL) by percolation at room temperature for 48 h. The gel was soaked and macerated in methanol (1 g/ 2 mL) by stirring for 48 h. The extraction processes were repeated thrice for each sample and the resultant solubles were concentrated on rotary evaporator at 40 ^{o}C to afford the respective extracts. In total forty five methanolic extracts from fifteen *A. vera* samples have been generated and their yields are presented in Table 1.

Standard Aloin A

Aloin A, the major bio active molecule of *A. vera* , isolated earlier in our group from the

Table 1. Methanolic extracts (%) of *Aloe vera* plant accessions collected from different geographical areas of India

Area of collection	Sample code	Plant part	Methanolic extract (%)
Adilabad, Telangana	AV1 a	Gel	0.84
	b	Leaf peel	7.43
	c	Root	14.98
Kadiri, A.P	AV2 a	Gel	0.44
	b	Leaf peel	15.73
	c	Root	73.87
CIMAP, (Tissue culture)	AV3 a	Gel	0.29
Hyderabad, Telangana	b	Leaf peel	16.12
	c	Root	74.90
CIMAP, (Mother plant)	AV4 a	Gel	0.05
Hyderabad , Telangana	b	Leaf peel	1.28
	c	Root	16.12
Prakasham, A.P	AV5 a	Gel	0.15
	b	Leaf peel	3.16
	c	Root	21.66
YSR Horticultural	AV6 a	Gel	2.05
University, Hyderabad,	b	Leaf peel	21.55
Telangana	c	Root	32.90
Khammam, Telangana	AV7 a	Gel	1.18
	b	Leaf peel	22.61
	c	Root	23.07
NBPGR, Jodhpur, Rajasthan	AV8 a	Gel	0.95
	b	Leaf peel	6.98
	c	Root	12.08
Tirupathi, A.P	AV9 a	Gel	0.75
	b	Leaf peel	15.60
	c	Root	56.87
Kurnool, A.P	AV10 a	Gel	0.31
	b	Leaf peel	11.81
	c	Root	39.86
Karimnagar, Telangana	AV11 a	Gel	0.47
	b	Leaf peel	34.29
	c	Root	61.37
NBPGR-Hyderabad,	AV12 a	Gel	1.00
Telangana	b	Leaf peel	33.77
	c	Root	91.54
Medak, Telangana	AV13 a	Gel	0.34
	b	Leaf peel	25.68
	c	Root	62.69
Rajahmundry, A.P	AV14 a	Gel	0.07
	b	Leaf peel	0.71
	c	Root	19.20
Guntur, A.P	AV15 a	Gel	0.14
	b	Leaf peel	17.50
	c	Root	80.87

methanolic extract of *A. vera* gel by column chromatography as bright yellow needles from methanol, m.p. 150 ^{o}C, R_f : 0.5 (ethyl acetate – methanol – water : 8:1:1.1: 0.8) was used as reference standard.

Insects

100 pairs of adult *S. oryzae* were introduced into jars of 1.0 L capacity containing 500 g of maize. The jars were covered with muslin cloth and fixed with rubber band to allow aeration and to prevent escape of weevils. The culture was maintained at a temperature range of 27 ± 2°C and relative humidity of 65 ± 5 %.Seven days after oviposition, all parent weevils were removed from each jar and were placed on another set of seeds kept at the same conditions. Removal of parent weevils and placement on a fresh seed medium repeated until sufficient numbers of laboratory reared weevils of known age are available. Five to seven day old adult weevils were used for experiments.

Bioassay

All 45 extracts made from fifteen different accessions of *A. vera*, were preliminary screened for insecticidal activities against adults of *S. oryzae*. The effectiveness of nine samples of methanolic extracts of *A. vera* were tested again for contact and repellent activities against *S. oryzae.* Aloin A, the major metabolite of *A. vera*, was used as reference standard for these experiments (0.002% w/v).

Repellency

Petri dish choice bioassay described by Talukder and Howse (1993) was conducted for assessing repellent activity. Filter papers (Whatman No 1, diameter 9 cm) were divided into two equal halves. One half was impregnated with 0.5 mL of aloin A or plant extracts with 0.02% and 5% w/v concentration respectively, while the other half of the filter paper was treated with only methanol (0.5 mL). The treated paper disks were air dried under fan and then placed inside a petri dish. Thirty unsexed adult insects were released in each petri dish and the lid was sealed with parafilm. The number of insects on each half of the paper was counted after 1, 5 and 24 h of exposure. The experiment was replicated five times in dark under 26 ± 1 ^{o}C, 60 ± 5% R.H. The data was expressed as percentage repulsion (PR) using the following formula: PR (%) = (Nc – 50) × 2,

Where Nc is the percentage of weevils present in the control half.

Contact toxicity

The contact toxicity of plant extracts were determined by filter paper impregnation method described by Kim *et al*. (2003). An aliquot of 1 mL of 0.02% aloin A or 5% w/v plant extracts dissolved in methanol was applied on Whatman No 1 filter papers of 9 cm diameter, which gave 0.0024 mg/cm^2 and 0.60 mg/cm^2, respectively. Control was setup with same volume of methanol without sample. After drying, each filter paper was placed at the bottom of petri dish and then twenty adults of *S. oryzae* were released in each of the dishes which were then covered with the lids. Five replicates were set for each treatment. The number of dead insects was recorded after 14days. The mortality was assessed by means of direct observation and when no leg or antennal movements were observed, the insects were considered dead. The experiment was carried out under ambient conditions. Per cent mortality was calculated by using the formula (Danga *et al*., 2015).Percentage of mortality (%) = (Number of dead insects / No of insects introduced) × 100.

LC Analysis

Instrumentation

The UPLC system consisting of two LC-20AD pumps, SPD-M20A diode array detector, DGU-20A$_3$ degasser and SIL-20AC HT auto sampler (all from Shimadzu, Kyoto, Japan) was used. The chromatographic data was recorded using an Acer (Xizhi, New Taipei, Taiwan) computer system with Lab-Solution (5.41.240 Version) data acquiring software (Shimadzu, Kyoto, Japan). Vortex shaker, sample tubes and repeater (Tarsons, Chennai, India) were used in the analysis.

Chromatographic Conditions

After several trial runs, chromatographic separation was accomplished on Waters XBridge C_{18} Column (150 × 4.6 mm, 3.5 µm) (Massachusetts U.S.A) under isocratic mode

of elution. The mobile phase was a mixture of acetonitrile (Merck):10 mM ammonium acetate buffer (Merck) (60:40, v/v) with pH adjusted to 3.0. The mobile phase was freshly prepared, filtered through a Millipore filter paper (pore size 0.45 µm) and degassed continuously by an on-line degasser. Separation was performed at room temperature using 1.0 mL/min flow-rate and 10 min run time. The injection volume and the detection wavelengths were set at 20 µl and 305 nm respectively. The chromatography and the integrated data were recorded by using an Acer (Xizhi, New Taipei, Taiwan) computer system using Lab-Solution (5.41.240 Version) data acquiring software (Shimadzu, Kyoto, Japan).

Preparation of Standard Solution

A quantity of 1.0 mg of aloin A was taken in 10 mL of volumetric flask and made upto the mark with acetonitrile. From this 100 ppm solution, different dilutions such as 50 ppm, 25 ppm, 5 ppm, 2 ppm, 1 ppm were made with acetonitrile and used for calibration. (Working standards of aloin A were prepared by appropriate dilutions of standard solution with acetonitrile). Different concentrations (1 ppm ~ 100 ppm) ? of standard were injected to generate the calibration curve. The calibration curve is plotted with concentration on X- axis and area on Y- axis.

Limits of Detection (LOD) and Quantification (LOQ)

Limits of detection (LOD) and quantification (LOQ) represent the concentration of the analyte that would yield signal-to-noise ratios of 3 for LOD and 10 for LOQ, respectively. LOD and LOQ were determined by measuring the magnitude of analytical back ground by injecting a blank and calculating the signal-to-noise ratio for each compound by injecting a series of dilute solutions of respective standard. In order to see the effect of aloin A on the repellency and contact toxicity against *S. oryzae*, the six extracts exhibiting highest activities have been subjected to detailed UPLC studies.

Statistical analysis

The per cent repellency and mortality values were transformed to arc sine before subjected to Analysis of variance (ANOVA) to correct for heterogeneity of varience. ANOVA were performed with SAS 9.3 version by using general linear model (GLM) (SAS Institute, 2008). The differences among treatment means were compared using TUKEY test.

RESULTS AND DISCUSSION

Repellency of plant extracts

A total of 45 extracts prepared from fifteen different accessions of *A. vera*, were preliminary screened for insecticidal activities against adults of *S. oryzae.* Out of them, only nine samples of methanolic extracts of *A. vera* exhibited considerable potency which might be due to the presence of bioactive compounds. The close analysis of data (Table 2) reveals that the repellency values varied with the type of plant extracts and aloin A.

Table 2. Repellency of different methanolic extracts (5% w/v) of *Aloe vera* and aloin A (0.002% w/v)against *Sitophilus oryzae* after 1, 5 and 24 h

Sample Code	% Repellency		
	1 h	5 h	24 h
AV 2b	48.0 ± 5.03^{AB}	61.6 ± 5.83^{CD}	60 ± 9.19^{BC}
AV 2c	21.3 ± 4.02^{D}	81.3 ± 2.30^{AB}	77.3 ± 2.47^{AB}
AV 6c	$34.7 \pm .22^{BCD}$	64 ± 5.96^{BCD}	61.3 ± 5.06^{BC}
AV 7b	28 ± 11.47^{CD}	62.7 ± 6.89^{BCD}	62.7 ± 2.17^{BC}
AV 9b	46.7 ± 3.85^{AB}	78.7 ± 7.28^{ABC}	78.7 ± 4.99^{AB}
AV9c	44.0 ± 4.45^{ABC}	71.7 ± 3.26^{BCD}	61.4 ± 14.11^{BC}
AV11a	48.0 ± 5.77^{AB}	81.3 ± 4.08^{AB}	82.7 ± 2.82^{A}
AV12a	37.3 ± 5.97^{BCD}	53.3 ± 3.84^{D}	53.2 ± 2.72^{C}
AV13b	50.7 ± 3.44^{AB}	65.3 ± 1.05^{BCD}	74.7 ± 2.02^{AB}
AloinA	64 ± 2.19^{A}	90.7 ± 3.51^{A}	85.2 ± 2.26^{A}

Mean (± SE) of five replicates of 30 insects each. P ≤0.001; Values followed by the same letter in a column do not differ significantly Tukey 's Multiple Range Test ($P \leq 0.05$).

The aloin A showed maximum repellency of 64.0, 90.7 and 85.2 % significantly at 1, 5 and 24 h after exposure, respectively compared to the remaining plant extracts. Among the extracts analyzed, AV 11 a and AV 2 b both exhibited maximum repellency of 48 % at 1 hr on *S. oryzae*, while a minimum of 21.3% repellency was observed AV 2 c (df = 9; F value 8.60; p = <0.0001) at the same time

interval. At 5 h after exposure, both AV 11 a and AV 2 c showed a maximum of 81.3% (df = 9; F value 7.39; p = <0.0001) followed by AV 9 b . Even at 24 h after exposure, the same trend was observed. The maximum repellency of 82.7 % was observed for AV 11 a (df = 9; F value 6.30; p = <0.0001) followed by AV 9 b, AV 2 c and AV 13 b. In samples AV 6 c, AV 9 c, AV 2 b, AV 7 b exhibited repellency to *S. oryzae* in the range of 60 to 62.7 %, while the lowest was recorded for AV 12 a.

The present study showed definite impact of *A. vera* plant extracts with some variations towards inducing repellent activity to *S. oryzae*. As far as our knowledge is concerned, the present work represented the first report of its repellent and insecticidal activities of *A. vera* plant extracts against *S. oryzae* . Among the plant extracts tested, AV 11 a (gel) and AV 9 b (leaf peel) collected from Karimnagar, Telangana and Tirupathi, A.P showed maximum repellency against *S. oryzae*. However, AV 12 a (gel) and AV 2 b (leaf peel) from NBPGR, Telangana and Kadiri, A.P, respectively showed considerable repellency which indicated variation of biological properties with the geographical location of *A. vera*. Similarly, root extracts of *A. vera* also showed average repellency and varies with the location. The present results indicated that there is variation in repellency with locations which might be due to different phytochemical constituents and period of harvesting (Bougherra *et al*. 2015). The per cent repellency of *S. oryzae* increases at 5 h after exposure in all the plant extracts. The gel extract from Karimnagar and leaf peel from Medak showed increased repellency even at 24 h after exposure.

Contact toxicity of plant extracts

Analysis of the data on the effect of percentage mortality of various plant extracts on *S. oryzae* is presented (Table 3). Considerable mortality was observed among plant extracts except AV 11 a (gel) and AV 2 c (root). The per cent mortality of *S. oryzae* among the plant extracts ranged from 6.0 to 56.0 % at 14 days after treatment (df = 10; F value 45.36; p = <0.0001). The aloin A exhibited 58% mortality, while AV 13 b was able to induce 56.0% mortality. Samples AV 7 b and AV 9 b showed moderate toxicity (48.0%) to *S. oryzae* followed by AV 12 a (46.0 ± 3.16%) and AV 2 b (46.0 ± 3.16%). The lowest toxicity was observed for AV 11 a (6.0 ± 1.01%) and AV 2 c (10.0 ± 2.58%). No mortality was observed in the control.

The aloin A followed by AV 13 b (leaf peel) exhibited considerable mortality of *S. oryzae* compared to the remaining plant extracts. Irrespective of locations, AV 13 b (leaf peel) exhibited pronounced contact toxicity towards *S. oryzae*. Oparaeke and Kuhiep (2006) reported toxicity of *A. vera* leaf powder to *S. zeamais* at 20 % w/w and suppressed progeny development and grain damage. The probable reason for insecticidal activity is due to the presence of aloin, saponin in the leaf peel of *A. vera*. The exact mechanism for toxicity is not clear but might be due to physical abrasion of insect cuticle with consequent loss of body fluid and blockage of spiracle (Ogunwolu *et al*. 1998).

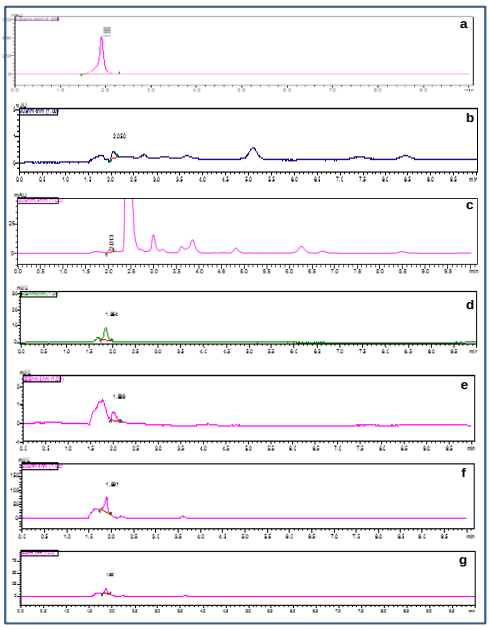


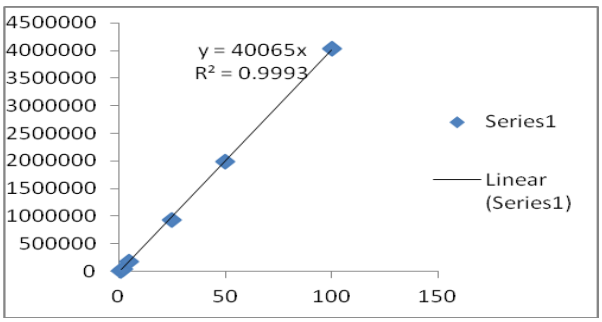
Figure 1. UPLC Chromatograms of (a) aloin A (b) AV 2 c (c) AV 7b (d) AV 9b (e) AV 11a (f) AV 12a (g) AV 13b

Chromatograms

Forty five different methanolic extracts of *A. vera* collected from different localities of Telangana, Andhra Pradesh and Rajasthan were screened against *S. oryzae* for their insecticidal activities. Active component aloin A was identified by LCMS.

Determination of Aloin A content in *Aloe vera* active extracts by UPLC

Typical chromatograms of aloin A and different plant parts of methanol extracts of *A. vera* are given in figures 1 A, B, C, D, E, F and G. A calibration plot was generated by using known authentic sample (Fig. 2). LOD and LOQ values are 0.3 ppm and 0.9 ppm respectively. The content of aloin A was determined for the five extracts and presented in Table 3.



X axis - Concentrations of samples ; Y axis - Areas of the samples

Fig. 2. Linearity curve of aloin A.

Impact of aloin-A content in methanolic extracts of *Aloe vera*

The per cent aloin A present in AV11 a (gel) was 0.07 (Table 3.) revealing clear repellent activity and minimum toxicity. AV 12 a (gel) contains highest amount of aloin A (6.54 %) showed minimum repellent activity. The per cent aloin A present in AV7 b, AV9 b (leaf peel) ranges from 0.2 to 0.9 showing considerable contact toxicity while AV2 c (root) contains 0.03 % aloin which showed less toxic effect. The results of the present study indicated the presence of aloin is responsible for contact toxicity to *S. oryzae*. Repellent activity of *S. oryzae* could possibly be due to the presence of other compounds such as terpenoids (Liang *et al*., 2013; Obeng-oferi *et al*., 1997). The present result is in agreement with Isarankura (1992) who reported that aloin isolated from *Cassia fistula* was toxic to *T. castaneum.* Aloin can be used as laxative, diet supplementary, anti inflammatory, antimicrobial and causes cellular modulation and immunological alterations.

Table 3. Accumulation of aloin A (%) in six active extracts of *A. vera*.

Sample Code	Activity	Aloin A (%)
AV2c	Lowest Toxicity	0.03
AV7b	Highest Toxicity	0.27
AV9b	Highest Toxicity	0.92
AV11a	Highest Repellency	0.07
AV12a	Lowest Repellency	6.54
AV13b	Highest Toxicity	1.69

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