The Pharma Innovation Journal 2019; 8(8): 154-159

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(8): 154-159

www.thepharmajournal.com
Received: 13-06-2019
Accepted: 15-07-2019

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Evaluation of plant extracts against rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae) in stored sorghum seeds

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Abstract
Botanicals severely in the stored product pest management. The present investigation was carried out to identify the suitable alternative plant extracts. There were seven pesticidal plant species selected and their leaves or plant parts having pesticidal effects the target insect *Sitophilus oryzae.* The maximum mortality of *S. oryzae* was observed with sweet flag rhizome (3%) extract (100%) and custard apple leaf extract (96.6%). The maximum repellency was observed sweet flag rhizome extract (80.00%) followed by chillies dry fruit extract (76.66%). The maximum fumigant toxicity action increase in sweet flag rhizome extract (95.00%) at 72 h after treatment. The effect of plant extract on germination of seed was also observed by germination test.

Keywords: Plant extracts, *Sitophilus oryzae*, sorghum seeds

Introduction
Sorghum, *Sorghum bicolor* L. is called as 'Camel of crops' because of its hardiness and ability to withstand prolonged droughts. The crop plays a major role in the food security of millions of people in marginal agricultural areas. It is the fifth most important crop in the world after rice, wheat, corn and barley. It is produced in hot region with a minimum temperature of 25°C to ensure maximum grain production. It accounts for an area of 5.65 million ha with production of 4.41 million tonnes in India (Pattanayak, 2016) [11].
The rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) is a major pest of stored sorghum in India and has been spread worldwide by commerce. This pest infests varieties of stored grains, cosmopolitan in distribution and more injurious in warm humid conditions. Both, the adults and larvae are voracious feeder on a different variety of grains and seeds, like sorghum wheat, corn, oats, rye, barley, rice and dried beans (Campbell, 2005.) [2] Suleiman *et al*. (2017) [14] reported an annual 13.12 per cent weight loss of threshed sorghum and 8.34 per cent of unthreshed sorghum.
Seed is the most vital and important input for crop production. It is necessary to protect the seeds through locally available plant products, which are eco friendly too. The plant kingdom is a rich store house of chemicals, which can prohibit the pest activity particularly in tropics and sub-tropics due to their insecticidal properties and some are with medicinal properties (Ileke and Oni, 2011) [6]. The plant extracts used as bio fumigants are not hazardous to human, easy to handle and safer to the environment (Kathirvelu and Raja, 2015) [8] In bulk storage, the storage pests can be controlled using plant leaves either as plant extracts or essential oil or applied as fumigants (Hashim *et al*., 2017) [4].

Materials and Methods
Maintenance of stock culture
The stock culture of the rice weevil was maintained by collecting adult weevils from the infested sorghum seeds from the godown. The rice weevils were reared in plastic containers of 1kg capacity containing the sorghum seeds under laboratory conditions at 27 ^{0}C and70% RH in continuous darkness. The mouth of container was covered by kada cloth fastened with rubber band. Fresh sorghum seeds were provided periodically for development of rice weevil and test insects were drawn from the stock culture for various investigations.

Plant extracts

The soxhlet extraction method was used in this study to extract more active principles from all the plant products. The dried plant materials (20g) of each plant species was filled into the Soxhlet apparatus. The required solvent (acetone) was filled up 1:5 ratio of the sample material into the flask of the apparatus. The temperature of the heating mantle was maintained at 60-65 °C (boiling point of acetone). The extraction was carried out for 5 to 6 h for each sample. The extract was transferred to the beaker and excess solvent was allowed to evaporate. The evaporated material was taken in a conical flask and covered with aluminium foil (Kathirvelu and Raja, 2015) [8]

Effect of plant extracts in the management of rice weevil

Toxicity test

The toxicity test of plant extracts was performed in the plastic containers (7.0 x 5.5 cm) containing 10g of sorghum seeds treated with 1 ml of 3% (w/v) concentration of leaf extract and their mixture. The adult weevils (10 pairs) of uniform size and age were introduced in to the containers. Same numbers of insects were kept in untreated sorghum seeds as control. The adult mortality was recorded from each replication at 3, 5, 7, 9 and 15 days after treatment.

Repellency test

The effect of plant extracts on the repellent action was evaluated based on the technique described earlier (Talukdar and Howse, 1993). A filter paper of 9 cm dia. was cut in half and 1 ml of 1% and 3% (w/v) concentration was applied separately to one half of the filter paper and the other half treated with 1 µl of acetone as uniformly as possible with a micropipette. Both the halves were then air dried to evaporate the solvent completely and a full disc was carefully remade by attaching them with an adhesive tape. A control was setup by placing acetone treated filter paper disc on a petri dish. The adult weevils (10 no.) were released in the centre of each filter paper disc and covered with a lid. Vaseline was applied on the inner vertical side of the Petri dish to prevent the weevils from climbing onto the side and lid of the Petri dish. Care was taken to see that the adhesive tape attachment did not prevent free movement of weevils from one half to another. The distance between the filter paper halves remained sufficient to prevent seepage of test samples from one half to another. The entire experimental setup was replicated thrice. Observations on the number of weevils settling on each filter paper were recorded at hourly intervals *viz*, 1, 2, 3, 4, 6 and at 24 h. The per cent repulsion (PR) values were computed as follows: PR = [NC-NT]/NC+NT x 100; where PR- = per cent repellency; NC = weevils present on acetone strip; NT = weevils present on treated strip.

Fumigant toxicity of botanical extracts against *Sitophilus oryzae* L.

The fumigation chamber was designed by using a plastic jar of 350 ml capacity provided with a screw lid (Rahman and Schmidt, 1999) [12] A circular filter paper (5 cm dia.) was pasted on the inner surface of the lid with adhesive tape. The adult weevils (10 no.) of uniform size and age were confined in vials (25 ml) and mouth of the vials were covered with net (25 mesh) to prevent weevil from escaping. The vials were placed inside the plastic jars. The 100 µl, 200 µl, 300 µl extracts and solvent (acetone) were applied on the filter paper by using micropipette and the lid was closed and sealed by adhesive tape to create airtight condition in the chamber and as three replications. One fumigation chamber without botanical extract was considered as control. Observations on the adult mortality were taken at 48 and 72 hours after treatment. The test insects showing any movement were considered to be alive.

Effect of plant extracts on the germination of sorghum seeds

Experiments on the germination of seed were carried out employing roll towel method, described by International Seed Testing Rules Association (ISTA) Manisha *et al*, (2015). Germination test was conducted at monthly interval for two months.

The paper was cut into a convenient size to hold one replicate of the seeds. The paper was labeled at one end with the accession number, replicate number and the testing date. A pencil or permanent marker was used for labeling. The paper was moistened with water. The seeds were arranged in rows at regular intervals about 4 cm from the top edge, leaving 3-4 cm gap on the sides. The seeds were covered with another sheet of moist paper towel. The paper was rolled loosely from opposite end to the label end. A paper clip or rubber band was used to hold the rolled papers to prevent unrolling. The rolls were kept upright in a deep-bottom plastic tray. Sufficient quantity of water was added to the tray. The trays were incubated at room temperature and the test was carried out for the recommended period (14 days). The towels were kept moist by spraying with water if necessary when temperature was high. The germinated seeds were counted by unrolling the paper carefully to avoid tearing or damaging the roots of young seedlings. The test was continued until all the seeds had germinated or until no further germination had occurred after two consecutive counts. The seeds that did not germinate were noted but they were firm and sound at the end of the first count and those that failed to germinate was presumed dead at the end of the germination test. Observations on the germination, seedling root length, shoot length and biomass were recorded.

Estimation of weight loss

The seed weight loss was estimated using count and weight method taking 100 sorghum seeds randomly from each replication from all treatments.

$$\text{Weight loss} = \frac{(Wu \times Nd) - (Wd \times Nu)}{Wu \times (Nd + Nu)} \times 100$$

Where Wu = Weight of undamaged seeds
Nu = Number of undamaged seeds
Wd = Weight of damaged seeds
Nd = Number of damaged seeds

Statistical analysis

The data obtained from the experiments were statistically analyzed by one factor CRD with the help of computer based program AGRES software after arcsine transformation.

Results

Toxicity test of botanical extracts (3%)

The sweet flag rhizome extract (100%) and custard apple leaf extract (96.6%) (3%) were equally effective in causing maximum mortality followed by tulasi leaf extract (66.66%)

lakke leaf extract (66.66%) while neem leaf extract (60.00%) and periwinkle leaf extracts (50.00%) on 3 DAT (Table 1). Subsequently, consistent results on mortality were observed in all the treatments throughout the period of observation. The maximum mortality was witnessed in sweet flag rhizome extract (100%) and custard apple extracts (96.66%) and minimum mortality in chillies dry fruit extract (40.00%). However, the control population also showed minimum mortality (6.66%) on 7 DAT.

Effect of botanical extracts on the repellency action against rice weevil

The plant extracts (3%) showed maximum repellency in sweet flag rhizome extract (80.00%) followed by chilies dry fruit extract (76.66%), neem leaf extract (73.33%), custard apple leaf extract (66.66%), periwinkle leaf extract (63.33%), lakke leaf extract (63.33%) and tulasi leaf extract (53.33%) within 1 h of exposure (Table 2). The repellency nature was consistent in all the plant extracts tested at varying intervals. The maximum repellency was attained at 24 h exposure in sweet flag rhizome extract (80.00%) and chillies dry fruit extract (80.00%) followed by other treatments.

Fumigant toxicity of botanical extracts against *S. oryzae*

Results on the fumigant action of plant extracts tested clearly showed that all the treatments possessed significantly superior toxicity in all the concentrations tested when compared to the control (Table 3). Among the different concentrations, botanical extracts at 300µl was found to possess maximum fumigant action when compared to the lower concentrations *viz.*, 100µl and 200µl. Among the treatments, sweet flag rhizome extract showed significantly superior toxicity (95.00%) at 72 h after treatment followed by periwinkle leaf extract (78.33%) and custard apple leaf extract (65.00%) on 72 h after treatment. However, the control also experienced minimum mortality of 1.66 and 5.00 per cent at 48 and 72 h, respectively.

Table 1: Effect of plant extracts (3%) on the mortality of rice weevil

Treatment	Dosage (ml /10g seed)	Mortality (%)*				
		3DAT	5DAT	7DAT	9DAT	15DAT
T1 – Tulasi leaf extract	1.0	66.66 (54.73)^{bc}	83.33 (65.90)^{c}	86.66 (68.58)^{c}	86.66 (68.58)^{bc}	86.66 (68.58)^{bc}
T2 – Lakke leaf extract	1.0	66.66 (54.73)^{bc}	80.00 (63.43)^{bc}	83.33 (65.90)^{bc}	83.33 (65.90)^{bc}	83.33 (65.90)^{bc}
T3 – Sweet flag rhizome extract	1.0	80.00 (63.43)^{a}	100 (90.00)^{a}	100 (90.00)^{a}	100 (90.00)^{a}	100 (90.00)^{a}
T4 – Neem leaf extract	1.0	60.00 (50.76)^{c}	63.33 (52.73)^{cd}	70.00 (56.78)^{cd}	73.33 (58.90)^{cd}	73.33 (58.90)^{cd}
T5 – Custard apple leaf extract	1.0	93.33 (75.03)^{a}	96.66 (79.48)^{ab}	96.66 (79.48)^{ab}	96.66 (79.48)^{ab}	96.66 (79.48)^{ab}
T6 – Periwinkle leaf extract	1.0	50.00 (45.00)^{cd}	66.66 (54.73)^{cd}	73.33 (58.90)^{c}	76.66 (61.11)^{cd}	86.66 (68.58)^{bc}
T7 – Chilli dry fruit extract	1.0	26.66 (31.09)^{d}	40.00 (39.23)^{d}	46.66 (43.08)^{d}	56.66 (48.83)^{d}	60.00 (50.76)^{d}
T8 - Control(acetone)	1.0	0.00 (0.62)^{e}	3.33 (10.52)^{e}	6.66 (14.96)^{e}	6.66 (14.96)^{e}	6.66 (14.96)^{e}
SED	-	7.76	7.57	6.81	7.24	8.01
CD(p = 0.05)	-	16.45	16.05	14.45	15.36	16.99

DAT – Days After Treatment

Figures in parantheses are arcsine transformed values

Means in a column followed by the same letters are not significantly different (p = 0.05) by LSD

*Mean of the replication

Effect of botanical extracts (3%) on the germination, seedling vigour, biomass and weight loss of sorghum seeds

Data on the quality parameters of sorghum seeds was much influenced by the effect of different plant extracts (3%) by inflicting minimum damage as evidenced by reduced seed weight loss when compared to control (Table 4). There was no reduction in seed weight loss in sweet flag rhizome extract treated seeds and custard apple leaf extract treatment, Chillies dry fruit extract and lakke leaf extract showed maximum weight loss followed by tulasi leaf extract, neem leaf extract and periwinkle leaf extract. The untreated check recorded the maximum weight loss due to weevil damage. The treatments influenced the other biological attributes of sorghum seeds *viz.*, germination, seedling length, biomass and vigour index. The maximum germination was observed in sweet flag rhizome extract (87.0%), custard apple leaf extract (80.0%), periwinkle leaf extract (76.0%), lakke leaf extract (75.0%), tulasi leaf extract (73.3%) followed by neem leaf extract (54.66%) and chillies dry fruit extract (49.3%). The germination was significantly affected in the untreated check (2.26%). Similarly, the seedling length was significantly increased in sweet flag rhizome extract (32.03 cm), custard apple leaf extract (31.09 cm) followed by periwinkle leaf extract (22.98 cm), tulasi leaf extract (21.34 cm), neem leaf extract (21.02 cm), lakke leaf extract (19.58 cm), chillies dry fruit extract (17.30 cm) and minimum in control (4.66 cm). The biomass production was maximum in sweet flag rhizome extract (2.30 g), custard apple leaf extract (1.87 g) and periwinkle leaf extract (1.35 g) and 0.02 g in control. The vigour index showed a significantly maximum in sweet flag rhizome extract (2786), custard apple leaf extract (2487) followed by periwinkle leaf extract (1746), tulasi leaf extract (1564), lakke leaf extract (1468),neem leaf extract (1149) and chillies dry fruit extract (853).

Table 2: Effect of plant extracts (3%) on the repellent action in rice weevil on sorghum seeds

Treatment	Dosage (µl /9 cm filter paper)	Repellency (%)*						Mean
		1 h	2 h	3 h	4 h	6 h	24 h	
T1 – Tulasi leaf extract	1.0	53.33 (46.91)^{b}	60.00 (50.76)^{b}	56.66 (48.83)^{c}	63.33 (52.73)^{b}	53.33 (46.91)^{c}	50.00 (45.00)^{c}	56.11 (48.52)^{e}
T2 – Lakke leaf extract	1.0	63.33 (52.73)^{ab}	66.66 (54.73)^{b}	70.00 (56.78)^{ab}	66.66 (54.73)^{ab}	63.33 (52.73)^{bc}	60.00 (50.76)^{bc}	65.00 (53.74)^{cd}
T3 - Sweet flag rhizome extract	1.0	80.00 (63.43)^{a}	83.33 (65.90)^{a}	86.66 (68.58)^{a}	86.66 (68.58)^{a}	80.00 (63.43)^{a}	80.00 (63.43)^{a}	82.77 (65.56)^{a}
T4 - Neem leaf extract	1.0	73.33 (58.90)^{ab}	70.00 (56.78)^{ab}	70.00 (56.78)^{ab}	73.33 (58.90)^{ab}	80.00 (63.43)^{a}	73.33 (58.90)^{ab}	73.33 (58.95)^{bc}
T5 - Custard apple leaf extract	1.0	66.66 (54.73)^{ab}	63.33 (52.733)^{b}	63.33 (52.73)^{bc}	56.66 (48.83)^{b}	50.00 (45.00)^{c}	50.00 (45.00)^{c}	58.33 (49.83)^{ed}
T6 – Periwinkle leaf extract	1.0	63.33 (52.73)^{ab}	70.00 (56.78)^{ab}	56.66 (48.83)^{c}	63.33 (52.73)^{b}	70.00 (56.78)^{ab}	73.33 (58.90)^{ab}	66.11 (54.46)^{d}
T7 – Chilli dry fruit extract	1.0	76.66 (61.11)^{a}	80.00 (63.43)^{ab}	83.33 (65.90)^{ab}	80.00 (63.43)^{ab}	76.66 (61.11)^{ab}	80.00 (63.43)^{a}	79.44 (63.07)^{ab}
T8 –Control (acetone)	1.0	0.00 (0.62)^{c}	0.00 (0.62)^{c}	0.00 (0.62)^{d}	0.00 (0.62)^{c}	0.00 (0.62)^{d}	0.00 (0.62)^{d}	0.00 (0.62)^{f}
SED		6.26	6.86	8.96	8.24	5.08	5.36	2.53
CD(p = 0.05)		13.27	14.56	18.99	17.48	10.77	11.37	5.36

h - hours
Figures in parantheses are arcsine transformed values
Means in a column followed by the same letters are not significantly different (p = 0.05) by LSD
*Mean of the replication

Table 3: Evaluation of plant extracts for fumigant action against *S. oryzae*

Treatment	Mortality (%)*					
	100 µl		200 µl		300 µl	
	48 h	72 h	48 h	72 h	48 h	72 h
T1 – Sweet flag rhizome extract	26.66 (31.09)^{a}	61.66 (51.74)^{a}	35.00 (36.27)^{a}	76.66 (61.11)^{ab}	71.66 (57.83)^{a}	95.00 (77.07)^{a}
T2 – Tulasi leaf extract	10.00 (18.43)^{c}	26.66 (31.09)^{c}	20.00 (26.56)^{cd}	40.0 (39.23)^{cd}	45.00 (42.13)^{bc}	61.66 (51.74)^{cd}
T3 - Lakke leaf extract	15.00 (22.78)^{bc}	28.33 (32.16)^{c}	26.66 (31.09)^{bc}	55.00 (47.86)^{b}	43.33 (41.16)^{bc}	58.33 (49.79)^{cd}
T4 - Neem leaf leaf extract	13.33 (21.41)^{bc}	25.00 (30.00)^{cd}	18.33 (25.35)^{d}	50.00 (45.00)^{bc}	46.66 (43.08)^{bc}	63.33 (52.73)^{cd}
T5 - Custard apple leaf extract	13.33 (21.41)^{bc}	33.33 (35.26)^{c}	23.33 (28.88)^{cd}	50.00 (45.00)^{bc}	48.33 (44.04)^{bc}	65.00 (53.72)^{bc}
T6 – Periwinkle leaf extract	20.00 (26.56)^{b}	46.66 (43.08)^{b}	31.66 (34.24)^{ab}	53.33 (46.91)^{b}	55.00 (47.86)^{b}	78.33 (62.25)^{b}
T7 – Chilli dry fruit extract	8.33 (16.77)^{c}	16.66 (24.09)^{d}	16.66 (24.09)^{d}	38.33 (38.25)^{d}	33.33 (35.26)^{c}	50.00 (45.00)^{d}
T8 - Control(acetone)	1.66 (7.41)^{d}	5.00 (12.92)^{e}	1.66 (7.41)^{e}	5.00 (12.92)^{e}	1.66 (7.41)^{d}	5.00 (12.92)^{e}
SED	3.30	3.22	2.42	2.80	4.34	4.15
CD(p = 0.05)	7.00	6.84	5.14	5.94	9.21	8.79

h - hours
Figures in parantheses are arcsine transformed values
Means in a column followed by the same letters are not significantly different (p = 0.05) by LSD
*Mean of the replication

Discussion

The bioassays carried out for assessing the insecticidal activities of plant extracts on *S. oryzae* showed that the sweet flag rhizome extract and custard apple leaf extract were equally effective in causing maximum mortality when compared to untreated check (acetone) in rice weevil 15 DAT. The sweet flag rhizome extract and custard apple leaf extract caused maximum mortality. Simultaneously, sweet flag rhizome extract and chillies dry fruit extract showed high repellent action. This study confirmed the results of Hossain *et al.*(2008) [5] who reported sweet flag acetone extract with 99 per cent mortality in *S. oryzae* on 24 HAT. Further, the insecticidal action of extracts from *A.calamus* was recorded 90 per cent mortality obtained at 3-4 days after treatment (Kim *et al*., 2003) [9]. The extract of *A. squamosa* showed complete mortality and produced significant "Knockdown" (KD50) 1 per cent w/v and 5 per cent w/v and being a contact poison for insects, can penetrate the body wall and tracheal system bringing about death probably lending the extract the insecticidal activity against *S. oryzae* (Ashok Kumar *et al.*, 2010) [1]. Iqbal *et al*. (2010) [7] reported that sweet flag acetone extract gave 93 per cent repellent action in *T. casteneum*. However, the sweet flag rhizome acetone extract showed minimum repellent action in *S. cerealella* (Iqbal *et al.*, 2010) [7]. In the present findings, chillies dry fruit extract showed high repellent action which coincided with findings of (Trematterra *et al.,* 2002) who reported chillies fruit extracts with maximum repellent action against *S. oryzae*.These

findings is well supported to the attractant / repellent compound from *Capsicum* employed in preventing or reducing the damage of *S. oryzae*.

Table 4: Effect of plant extracts (3%) on the biological parameters of sorghum seeds

Treatments	Dosege (ml /10g seed)	Weight loss* (%)	Germination* (%)	Seedling length* (cm)	Vigour index*	Biomass* (g)
T1 – Tulasi leaf extract	**1.0**	4.11 (11.70)^{bc}	73.3 (58.88)^{b}	21.34^{c}	1564^{d}	0.89 (5.41)^{cd}
T2 – Lakke leaf extract	1.0	6.80 (15.11)^{cd}	75.00 (60.00)^{b}	19.58^{c}	1468^{d}	0.84 (5.28)^{d}
T3 - Sweet flag rhizome extract	1.0	0.00 (0.62)^{a}	87.00 (68.86)^{a}	32.03^{a}	2786^{a}	2.30 (8.72)^{a}
T4 - Neem leaf extract	1.0	3.08 (10.10)^{b}	54.66 (47.67)^{c}	21.02^{c}	1149^{e}	0.57 (4.33)^{d}
T5 - Custard apple leaf extract	1.0	0.00 (0.62)^{a}	80.00 (63.43)^{ab}	31.09^{ab}	2487^{b}	1.87 (7.86)^{ab}
T6 – Periwinkle leaf extract	1.0	4.82 (12.68)^{bc}	76.00 (60.66)^{ab}	22.98^{bc}	1746^{c}	1.35 (6.67)^{bc}
T7 – Chillies dry fruit extract	1.0	7.66 (16.07)^{d}	49.3 (44.59)^{c}	17.30^{c}	853^{f}	0.63 (4.55)^{d}
T8 - Control(acetone)	1.0	17.07 (24.40)^{e}	2.66 (9.38)^{d}	4.66^{d}	12^{g}	0.02 (0.93)^{e}
SED	-	1.85	4.00	2.58	60.55	0.72
CD(p = 0.05)	-	3.92	8.49	5.47	128.38	1.54

Figures in parantheses are arcsine transformed values
Means in a column followed by the same letters are not significantly different (p = 0.05) by LSD
*Mean of the replication

In the present investigation, sweet flag rhizome extract and custard apple leaf extract treated seeds produced zero per cent weight loss and have good seed quality parameters when compared to untreated check after 60 days of storage (DAS). This study is in agreement to earlier reports that sweet flag rhizome extract and custard apple leaf extract treated seeds produced good germination and minimum weight loss. (Hossain *et al.*, 2008) [5] Ashok Kumar *et al.*,(2010) [1].

The present investigation on the fumigant action of plant extracts against *S.oryzae* proved maximum fumigant action by the sweet flag rhizome extract (95.0%) and periwinkle leaf extract (78.33%) on *S. oryzae* (Fig. 3). This study is accordance with earlier findings that *A. calamus* rhizome extract indicated fumigant action and caused 100 per cent mortality of *S. oryzae*(Kim *et al*. 2003) [9]. These results indicated the insecticidal mode of action of the oils and extracts which may be largely attributable to fumigant action, they may be toxic by penetrating the insect body *via* the respiratory system.

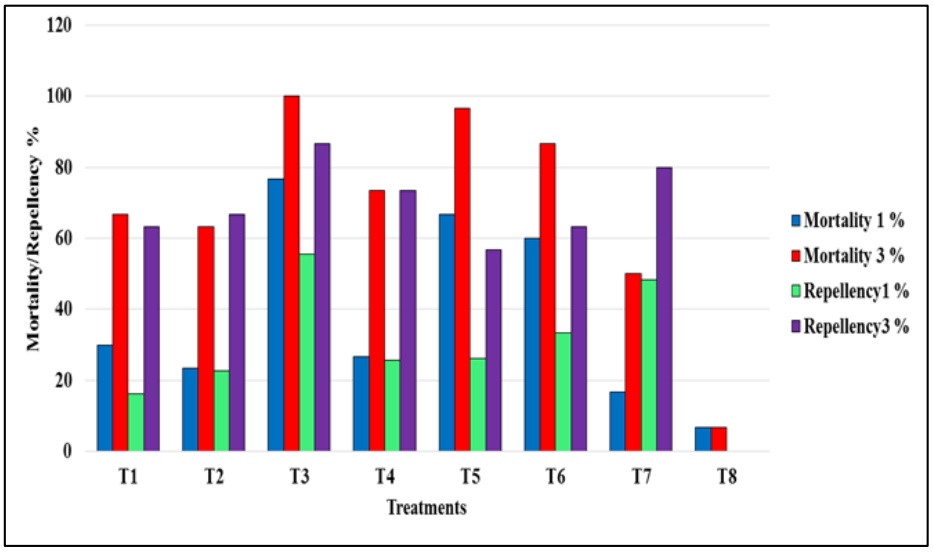

Fig 1: comparative analysis on the effect of plant extract on the morality and repellency against rice weevil

T1 – Sweet flag rhizome extract, T2 -Tulasi leaf extract, T3 - Lakke leaf extract, T4 - Neem leaf extract, T5 – Custard apple leaf extract, T6 - Periwinkle leaf extract, T7 – Chillies dry fruit extract, T8 – Control (acetone)

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