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Traditionally used Botanicals: The Potential Source of *Tribolium castaneum* (Herbst) Management

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Wheat is widely used as a staple food in the world including India. But storage conditions of wheat grains are infected by several coleopteran pests. *Tribolium castaneum* Herbst is a ubiquitous and pestiferous pest among all. Adult and larvae both are harmful to wheat storage which often demolishes stored grains. The present study attempted at Entomology laboratory, Assam Agricultural University-Jorhat, to manage Dry powders of *A. indica, C. roseus, C. heptaphylla, D. stramonium, E. tereticornis, M. struthiopteris,* and *V. negundo* were used against *T. castaneum* to get rid of problematic local medicinal plants. During probit analysis the highest LD_{50} value was found in *A. indica* (1.49%) followed by *D. stramonium* (1.52%) and *E. tereticornis* (2.02%) and the lowest LD_{50} in *Matteuccia struthiopteris* (11.72%). In the repellency test, the highest rate of repellency was observed in *A. indica* (82.21%) followed by *D. stramonium* (72.59%), *E. tereticornis* (70.36%), and the lowest in *Matteuccia struthiopteris* (32.58%). Based on LD_{50} and mean repellency, the three botanicals (*A. indica, D. stramonium, E. tereticornis*) were selected for further work e.g., mortality and weight loss. A mortality study recorded 100% mortality after 35 days of

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treatment in the case of *A. indica, D. stramonium, E. tereticornis*. During the trial in 2018, *A. indica* (5.76%) had the lowest grain weight loss, followed by *D. stramonium* (12.05%) and *E. tereticornis* (12.05). The highest grain weight loss was observed in control with 62.33 per cent. Give your one line conclusion on these research.

Keywords: Wheat; Tribolium castaneum; botanicals; LD_{50}; repellency.

1. INTRODUCTION

"Wheat is one of the most important cereal grains, used widely as staple food in many countries for high nutritive values. The population is expected to grow to 9.1 billion people by the year 2050, and about 70% extra food production will be required to feed those".[20] "However, one of the greatest constraints of maintaining wheat is insect infestation, including over 600 different kinds of beetles, 70 species of moths, 355 species of mites, 40 species of rodents, and 150 species of fungi invading wheat" [47]. Among them, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) is a ubiquitous and pestiferous storage pest which often demolish stored grains specially wheat causes 328mg weight loss due to infestation of adults and larvae [54]. Due to infestation of this insect flour turns grayish and mouldy with pungent odour [21,56,22].

"Finding more effective and healthy substitutes has become required due to synthetic insecticide toxicity and resistance issues. Presently, plant extracts are the most commonly tested alternative products to remove the stored grain insect pest" [51,66,19,14,67,]. Botanicals contain many alkaloids and other secondary metabolites which have insecticidal and anti-feedant properties against several storage pests [33,9,53,26,27,29]. Kumral *et al.,*[37] also described about the acaricidal, repellent, and oviposition deterrent activities of various botanicals *viz*., *Annona squamosa* (L.), *Moringa oleifera* (Lam.) and *Eucalyptus globulus* (Labill.) etc.

"To overcome the problems of synthetic pesticides, there is an urgent need for an eco-friendly, economically viable alternative pest control method that is affordable for poor farming communities, safe for worker's health, and has higher returns" [60,15,48,31,38]. Talukder [64] listed "43 plant species as insect repellents, 21 plants as insect feeding deterrents, 47 plants as insect toxicants, 37 plants as grain protectants, 27 plants as insect reproduction inhibitors, and 7 plants as insect growth and development inhibitors". "Among the medicinal plants, several domestic plant species have been reported to be repellent and toxic to *T. castaneum"* [42,49,63]. Therefore, the present study seven locally available and farmer used plants dry powders were tested against *Tribolium castaneum* (Herbst) in the Department of Entomology, AAU Jorhat- 13 in 2018.

2. MATERIALS AND METHODS

To find out the dry powder efficacy of seven botanicals *viz. Azadirachta indica, Catharanthus roseus, Clausena heptaphylla, Datura stramonium, Eucalyptus tereticornis, Matteuccia struthiopteris,* and *Vitex negundo* against *Tribolium castaneum* (Herbst). An experiment was carried out in 2018, in the Toxicology Laboratory of the Department of Entomology at AAU Jorhat-13.

"Adults stage of *Tribolium castaneum* (Herbst) were collected from Post-Harvest Laboratory, Department of Agricultural Engineering, Jorhat-13 for mass rearing" [1,2]. "The fresh wheat seed (HD- 3086) was provided for feeding in eight different plastic containers (2 No's 29cm×12cm, 2 No's 32cm×13.50, 2 No's 25cm×12cm, 2 No's 22cm×11cm). The insect culture was maintained in toxicology laboratory, Department of Entomology, AAU Jorhat- 13, on room temperature 27- 30^0C" [10].

"Leaves were collected and washed with tap water and kept in shade for air- drying. After complete drying, electric blender was used to make and fine powder and obtained by sieving through kitchen strainer" [5]. "For each plant leaf powder was used as 0.25, 0.50, 0.75, 1.00, 1.25, 1.75 and 2.50 gm / 25 g seed were thoroughly mixed which correspondence to 1, 2, 3, 4, 5, 7 and 10 per cent (wt./wt.)", [50]. The treated seeds were stored in plastic containers (7 cm × 6 cm) at room temperature (25±5^oC) with three replications and 20 adults for each treatment. After 24, 48, and 72 h of therapy, the mortality was calculated. If there was mortality in control, Abbott's formula [3] was used to rectify it. To get the LD_{50} value (wt/wt), probit analysis was used on the mortality data that was obtained.

"For repellency test, 9 cm diameter Petri dish was divided into three parts, treated, untreated and without grain part [32]. In treated part of the plate filled with 0.3g of dry powder from each plant were mixed with 3g of wheat seed (10% wt/wt)"[17].

Test chamber with one side treated and the other untreated. Each Petri dish middle area received ten insects, which were then released w hile being protected by a cover plate. Until the sixth hour, the number of insects on each chamber was counted hourly. The % repellency has been determined using the formula below-

PR (%) = (Nc-50) x 2

Where: PR= % repulsion,

Nc= Percentage of weevils present in the control half.

According to Mc Donald *et al.* [44], the grades of repellency (% repellency rate) were categorized. One ANOVA with the JMP SAS and IBM SPSS 20 packages was used to examine differences between distinct means.

Repellency rate (%) Class

> 0.01	-	0.1	0
0.1	-	20	I
20.1	-	40	II
40.1	-	60	III
60.1	-	80	IV
80.1	-	100	V

The four most effective botanicals were chosen from the LD_{50} testing. 10 g dry powder of these botanicals was mixed with 100 g (10% w/w) seeds [50]. The treated seeds were subsequently released along with 20 individuals (male and female, in a 1:1 ratio) to observe *T. castaneum* mortality. At 1, 3, 7, 14, 21, 28, 35, and 45 days, the number of dead insects was counted in order to assess mortality. Counting was stop after 45 days to avoid the overlapping generation [16]. If mortality was found in control subjected to percentage of corrected mortality by Abbott's [3].

The following formula was used to determine the weight loss of grains.

% WL= (IW-FW) X 100 / IW

Where, WL: Weight loss index.

IW: Initial weight and FW is the final weight.

3. RESULTS AND DISCUSSION

3.1 Dry Powders' Effectiveness in Combating *Tribolium castaneum*

In 2018, the comparative toxicity and LD_{50} of seven plant leaf powders were assessed. Relative lethality has been expressed as a percentage and LD_{50} has been expressed as a percentage with weight/weight g. The result of experiment of laboratory efficacy and relative toxicity were described below. After 24 hours of exposure, *A. indica, C. roseus, C. heptaphylla, D. stramonium, E. tereticornis, M. struthiopteris,* and *V. negundo* had LD_{50} values of 2.09, 8.15, 9.05, 2.97, 2.81, 19.40, and 11.72 percent (wt/wt) against *T. castaneum*, respectively. After 48 hr of exposure it was 1.71, 3.82, 3.48, 2.01, 2.49, 14.64, 3.94 per cent and after 72 hr 1.49, 2.71, 4.44, 1.52, 2.02, 4.45, 2.80 per cent respectively (Fig. 1).

The toxicity order of diverse plant powders against *Tribolium castaneum* (Herbst) was *A. indica* > *E. tereticornis* > *D. stramonium* > *C. roseus* > *C. heptaphylla* > *V. negundo* > *M. struthiopteris* when *A. indica* was considered as standard for the exposure period of 24 hr. After 48 hr and 72 hr of exposure, the order of toxicity was *A. indica* > *D. stramonium* > *E. tereticornis* > *C. heptaphylla* > *C. roseus* > *V. negundo* > *M. struthiopteris* and *A. indica* > *D. stramonium* > *E. tereticornis* > *C. roseus* > *V. negundo* > *C. heptaphylla* > *M. struthiopteris,* respectively (Fig. 1).

In the present investigation, *A. indica* was considered as standard to evaluate the relative toxicity of selected botanicals. After exposure for 24, 48, and 72 hours, respectively. The respective toxicities of *C. roseus, C. heptaphylla, D. stramonium, E. tereticornis, M. struthiopteris,* and *V. negundo* were found to be significantly less toxic than *A. indica* (Fig. 1). In bioassay study, it was perceived that the per cent mortality of *Tribolium castaneum* (Herbst) due to the botanicals action was raised gradually with increase in concentrations as well as exposure period.

During the experiment, it was discovered that the relative toxicity and LD_{50} of the plants *A. indica, C. roseus, C. heptaphylla, D. stramonium, E. tereticornis, M. struthiopteris,* and *V. negundo* varied significantly. Comparing these botanicals to other botanicals, *A. indica, D. stramonium,* and

E. tereticornis produced the best results. *A. indica* leaf powder was found to have the maximum toxicity against *T. castaneum* (Herbst) based on LD_{50} and relative toxicity, which was consistent with findings by Mamun *et al.* in 2009 [40] and Rehman *et al.* in 2019 [55]. Different authors described the toxicity of *A. indica* against numerous storage pests. They reported that the presence of triterpenoid /secondary metabolites azadirachtin, salanin, meliantriol Mbailao *et al*. [43], Ileke and Oni [25] were responsible for antifeedant, ovicidal.

Mortality based on 4 replications each with 20 individuals larvicidal, insect growth regulatory, and repellent activity [13] of *A. indica. Eucalyptus tereticornis* leaf powder was found effective against *T. castaneum* (Herbst) based on LD_{50} experiment. Earlier workers also observed that the eucalyptus was effective against *T. castaneum* [59] and *Trogoderma granarium* [5]. Jawalkar *et al.,* [30]; Al Bachchu; Jahan *et al.,* [6] also used *D. Stramonium* and *A. indica* against storage pests and found similar results.

3.2 Repellency Testing against *Tribolium castaneum*

The repellency rate was observed highest in *A. indica* (86.66%), which was significantly different from other treatments, followed by *D. Stramonium* (73.33%), *E. tereticornis* (73.33%), and *C. heptaphylla* (66.66%), which were statistically similar with each other. *C. roseus* (46.66%) showed a comparatively less repellency rate, while *V. negundo* (33.33%), *M. struthiopteris* (26.66%) gave significantly less repellency rate in comparison to other treatments. After 2 h onward the repellency rate gradually increased and was found highest in the case of *D. struthiopteris* (80.00%) after 72 hr of treatment. After 72 hours of treatment, there was no discernible difference in the repellency of *A. indica* (80.00%), *C. roseus* (33.33%), *C. heptaphylla* (60.00%), and *E. tereticornis* (73.33%). After 72 hours, *M. Struthiopteris* (40.00%) and *V. negundo* (40.00%) likewise recorded lower repellency rates (Fig. 2).

After observation of 1, 2, 3, 4, 5, 6, 24, 48, and 72 h the mean repellency rate was found in *A. indica* (82.21%) which was under the repellency class V (based on the scale given by Mc Donald *et al.*, [44] followed by *D. stramonium* (72.59%), *E. tereticornis* (70.36%), *C. heptaphylla* (64.07%) and *V. negundo* (53.33%) (class IV), while *C. roseus* (47.67%) was found with low repellency rate under class III. The less mean repellency was observed in case of *M. struthiopteris* (32.58%) under repellency (class II). There was no repellency observed in control treatment. (Fig. 2) After *D. stramonium, A. indica,* leaf powder shown greater repellency against *T. castaneum* (Herbst) in treated wheat grains. The significant toxicity of *D. stramonium* against storage pest was also noted in earlier works by various researchers [1,2,30]. Another study by Manzoor et al. [41] found that *D. stramonium* had the highest level of repellency toward *Callosobruchus chinensis*.

Hanif *et al.,* [23] also reported "the highest rate of repellency advocated by *A. indica* and *D. stramonium* (77.66%, 81.48% and 76.43%) against *T. castaneum*, *Rhyzopertha dominica* and *Trogoderma granarium"*. Many workers reported that *D. stramonium* has both poisonous and medicinal properties [61,45]. All parts of *D. stramonium* are toxic to mammals [45]. In Ayurvedic, *D. stramonium* is described [35,61] as remedial medicine for various human ailments. *Eucalyptus tereticornis* leaf powder found effective against *T. castaneum* (Herbst) based on repellency. Naseem and Khan [46] showed that higher concentration (60%) of *E. camaldulensis* recorded 75.83% repellency of *T. castaneum* after 3 h of treatment.

Zero and cent per cent values were subjected to the formula ¼ n before angular transformation after Steel and Torrie, [62] where n= number of insects.

3.2 Mortality of *Tribolium castaneum*

The results in the year 2018 revealed that *A. indica* dry leaf powder had given maximum (32.50%) mortality of *T. castaneum* followed by *D. stramonium* (31.25%) and *E. tereticornis* (28.75%), which were statistically similar to each other and significantly different from the control one day after treatment, while no mortality was found in control. Similarly, at 3, 7, and 14 days after treatment also, *A. indica* showed the highest mortality (42.50, 52.50 and 62.50%, respectively) than *D. stramonium* (40.00, 50.00 and 60.00%, respectively) and *E. tereticornis* (35.00, 40.00 and 55.00%, respectively) were shown to have a lower mortality rate than *A. indica*. At 21 and 28 days after treatment, it was observed that all the treatment's mortality increased gradually and was found 100% at 35 days after treatment. *A. indica* showed significantly highest mortality (80.00% and

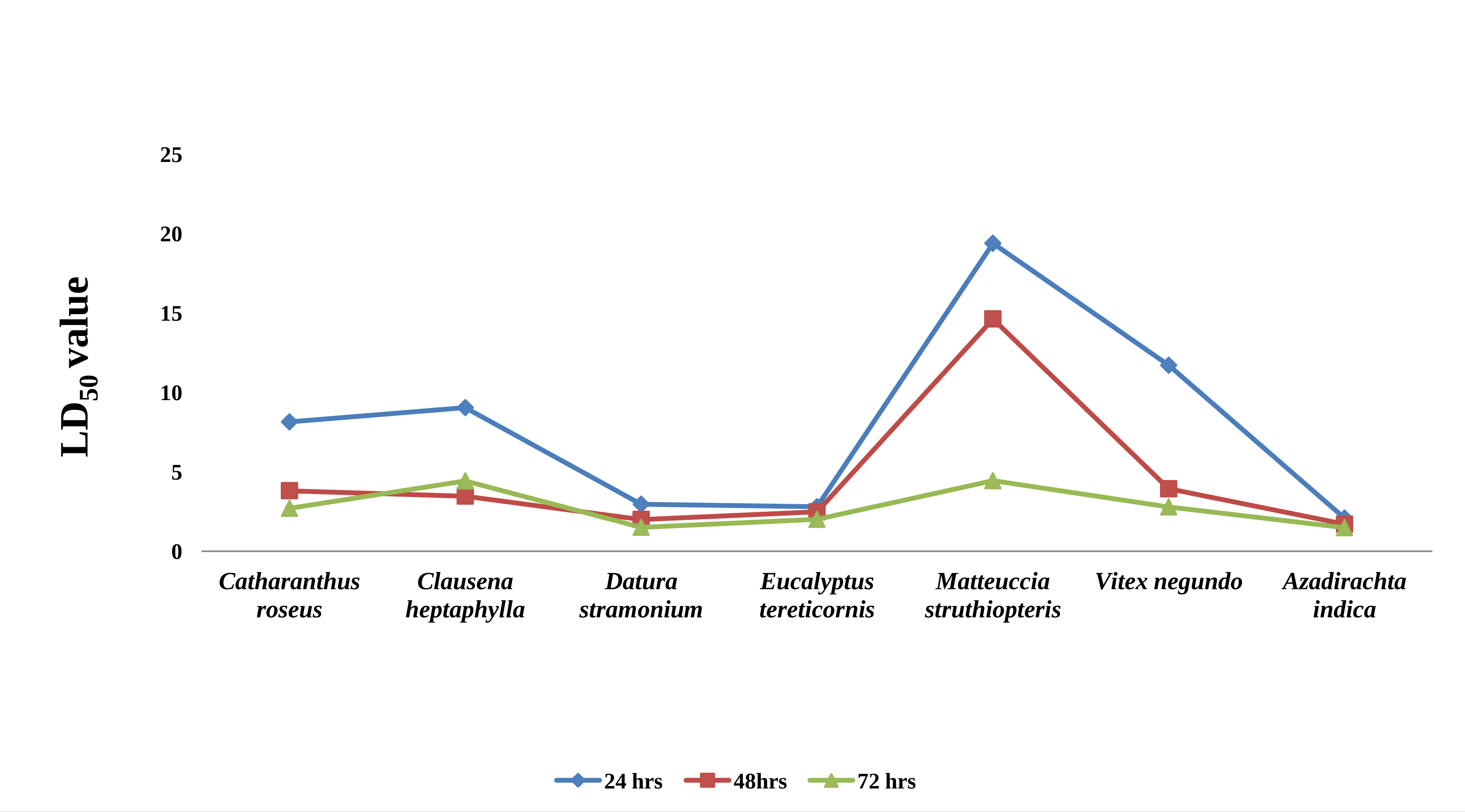

Fig. 1. LD_{50} and Relative toxicity of different plant powder against *Tribolium castaneum* (Herbst) after 24, 48 and 72 hours of treatment (2018)

The data were found to be significantly heterogeneous at = 0.05

Y = Probit kill, X = log dose

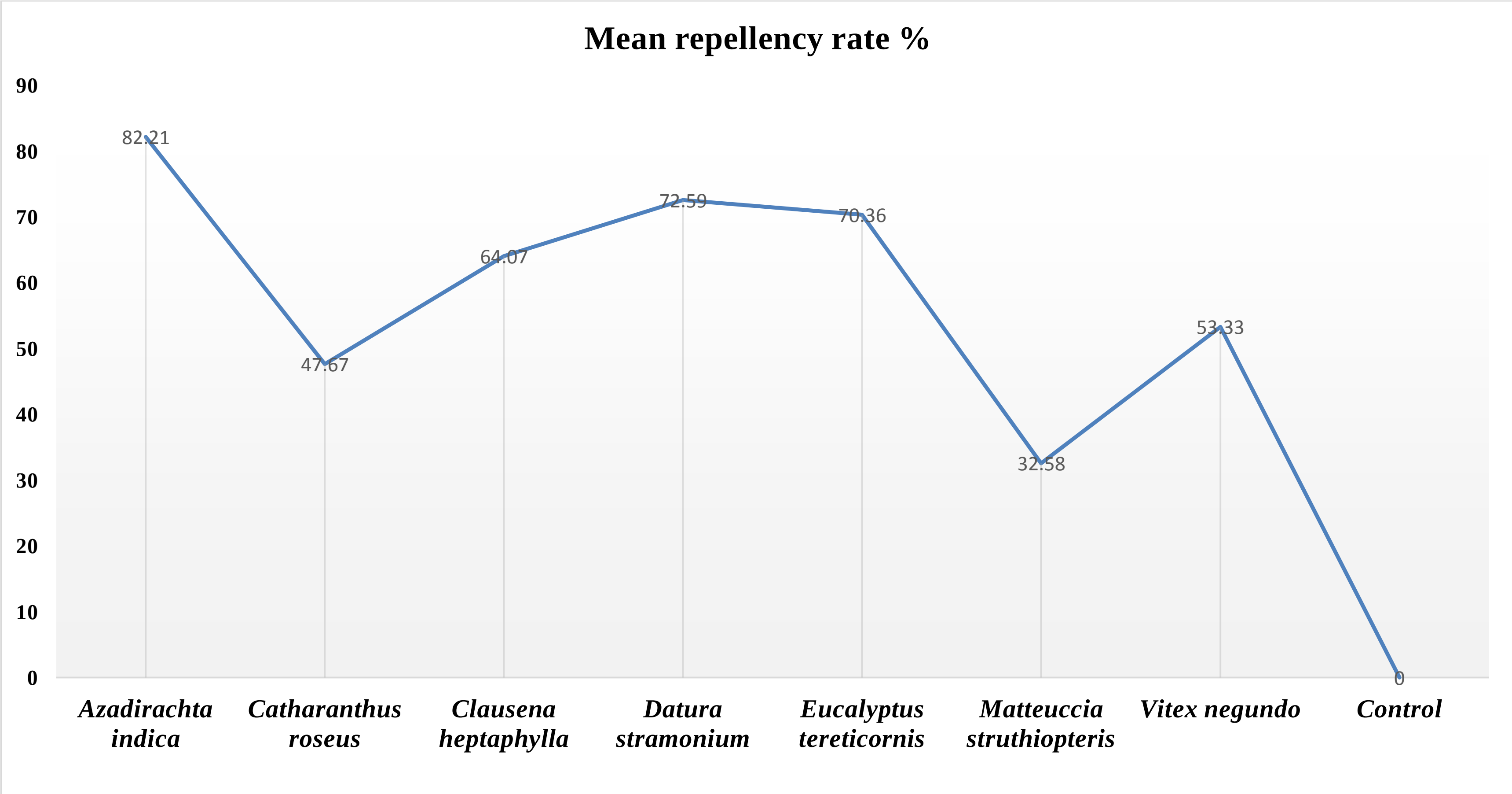

Fig. 2. In 2018, the effect of several plant leaf powders at repelling *Tribolium castaneum* (Herbst)

Figures with in parentheses are transformed values

Data are based on 3 replications each with 10 individuals

92.50%, respectively), followed by *D. stramonium* (77.50% and 90.00%, respectively) and *E. tereticornis* (75.00% and 87.50%, respectively) which were statistically at par. No mortality was observed in the case of control (Fig. 3). Effect of botanicals on stored grain pests was also observed in previous studies by Kumar *et al.* [36] and Perera and Karunaratne [52]. *D. stramonium* leaf powder also demonstrated increased mortality in treated wheat grains when compared to *A. indica* when compared to *T. castaneum* (Herbst). Previous works of many workers also reported the high toxicity of *D. stramonium* against storage pests [30,1,2]. Another study, by Manzoor *et al.* [41], discovered that *D. stramonium* caused the most mortality in *Tribolium castaneum*. Based on mortality, *Eucalyptus tereticornis* leaf powder was proven to be efficient against *T. castaneum* (Herbst). Earlier workers also observed that eucalyptus was effective against *T. castaneum* [59] and *Trogoderma granarium* [5]. *E. globulus* registered 100 percent mortality [57] and 71 percent [12] feeding inhibition activity *of S. oryzae.*

3.4 Weight Loss of Wheat Grains

All of the released insects died after 35 days when wheat grains were treated with *A. indica*, *D. stramonium*, and *E. tereticornis* to observe the mortality of *T. castaneum*.

So, 35 days later, the weight reduction of the grains was determined. The lowest grain weight loss was seen in *A. indica* (5.76%), followed by *D. stramonium* (12. 05%) and *E. tereticornis* (12. 05) throughout the experiment in 2018. *T. castaneum* discharged plant dry powder treated containers.

The control group experienced a significant grain weight decrease in 2018 (62.33%), which was significantly higher than that of the other botanicals treatments (Fig. 4). Most of the secondary metabolites are highly effective against insect pests, ecofriendly, easily extractable and biodegradable, with low or no mammalian toxicity. [65,28,7,8,58,4,11,39].

Most of the plant terpenoids showed repaid knockdown effect indicating neurotoxic mode of action [18]. Effect of botanicals on stored grain pests were also observed in previous studies by Kumar *et al.* [36] and Perera and Karunaratne [52]. *D. stramonium* leaf powder also demonstrated with higher weight loss results in treated wheat grains when compared to *A. indica* against *T. castaneum* (Herbst). Based on a weight loss experiment, *Eucalyptus tereticornis* leaf powder was proven to be efficient against *T. castaneum* (Herbst). The earlier weight loss studies [24,34] are also showing similarity with present study.

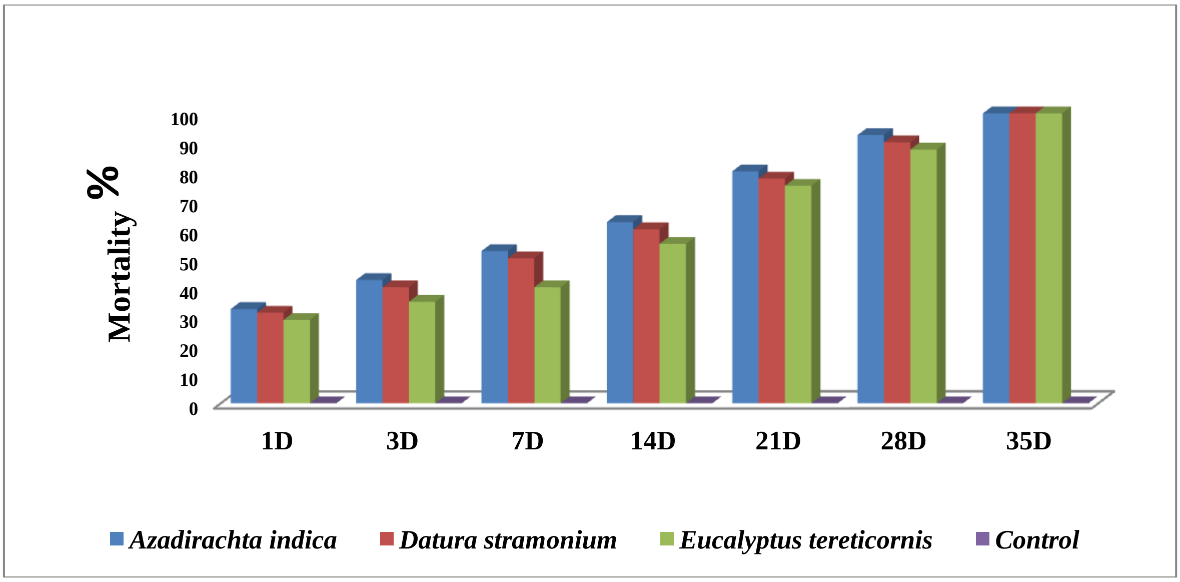

Fig. 3. Mortality of *Tribolium castaneum* (Herbst) after using different plants powder in 2018
D= Day after spraying
Figures are average corrected mortalities of 4 replications with 20 insects
Figures with in parentheses are transformed values
Zero and cent per cent values were subjected to the formula ¼ n before angular transformation (after Steel and Torrie, 1960), where n= number of insects

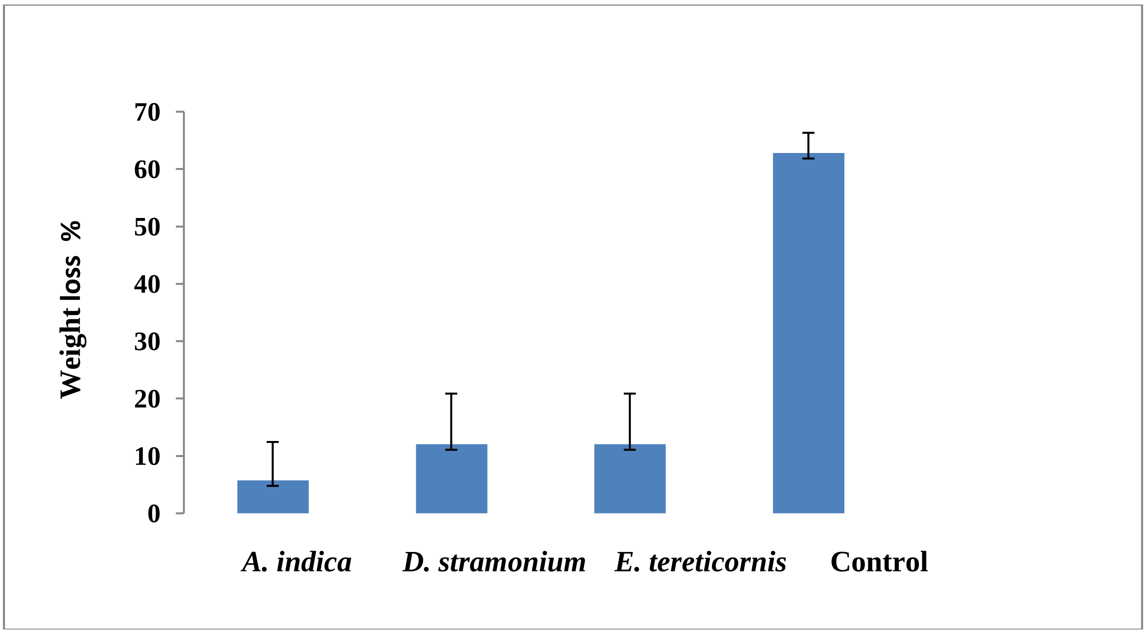

Fig. 4. Weight loss brought on by *T. castaneum* in wheat during 2018

4. CONCLUSION

This study concluded that *A. indica, C. roseus, C. heptaphylla, D. stramonium, E. tereticornis, M. struthiopteris,* and *V. negundo* are the most dependable, inexpensive, easily available, and excellent insecticide choices for combating *T. castaneum*.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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