

Cost effective natural treatment against infestation by *Tribolium castaneum* in stored food products

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Abstract

Introduction: The aim of this study was to test the repurpose of *Artemisia annua* Linn. dry leaf therapy (ALT) tablets of var. *Sanjeevani* and *Jeevan Raksha* for the control of *Tribolium castaneum*. **Methods:** 600 mg ALT tablets and 600 mg ALT + PSP (*Piper nigrum* seed powder) tablets (ALT powder and PSP in 5:1 proportion) were investigated to assess the survival and growth of *T. castaneum* feeding on wheat flour. **Results:** The progeny adults were found to be produced with about 57% and 38% less frequency in the jars containing ALT tablet of var. *Sanjeevani* and *Jeevan Raksha*, respectively, as compared to the control jars. Similarly, 12% of the insects died in the presence of ALT var. *Jeevan Raksha* tablet, the mortality was 18% higher in the presence of ALT var. *Sanjeevani* tablet. Likewise, 24% of the insects died in the presence of ALT var. *Jeevan Raksha* + PSP tablet, the mortality was 33% higher in the presence of ALT var. *Sanjeevani* + PSP tablet. The life cycle of *T. castaneum* was attenuated by var. *Jeevan Raksha* ALT and its ALT + PSP tablets in such a way that progeny size was reduced by these treatments as compared to control by 36% and 67%, respectively, whereas, var. *Sanjeevani* ALT and its ALT + PSP tablets reduced progeny size by 49% and 77%, respectively. **Conclusion:** It was found that var. *Sanjeevani* ALT + PSP tablets were more effective than other tablets against growth and survival of *T. castaneum* insect and thus provide a highly effective and low-cost means of controlling *T. castaneum* infestation on food materials, especially in an Indian household.

Key words: 1,8-cineole, *Artemisia annua*, f. Asteraceae, (cultivar-*Jeevan Raksha*, *Sanjeevani*), Black pepper, Red flour beetle

INTRODUCTION

The red flour insect species *Tribolium castaneum* (order, Coleoptera; family, Tenebrionidae) [Figure 1a] is found worldwide and is responsible for the decay of stored food materials on a large scale. It infests and spoils cereal grains, leguminous seeds, dry fruits, nuts, and other processed food materials such as pasta and biscuits. *T. castaneum* damages the grains and make them unfit for use. Natural hot and humid environmental conditions of tropical and semi-tropical regions and centrally heated conditions of enclosed areas in temperate regions are suitable for the infestation of insects in stored food products. The polyandrous female of the species lays 300–600 eggs at a time which passes through larval and pupal stages to emerge as an adult insect in about 4–24 weeks depending on the environment. The most suitable conditions

for the growth of *T. castaneum* are 25–35°C temperature, 50–70% relative humidity (R.H.), and ≤12 h exposure to light. The adult insects are known to survive for more than 3 years.^[1–3]

A variety of measures are already available to control *T. castaneum* infestation into food materials, but the available measures are ineffective to inhibit its resilience.^[4] Therefore, there is a need for an effective and new pest control method which is harmless to humans. Essential oils distilled from

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organs of certain plants having very low toxicity toward humans and used as fumigants offer a promising solution. Studies have identified 1,8-cineole (eucalyptol), and limonene and the essential oils containing them, such as those extracted from the shoot of *Artemisia annua* and/or peels of citrus fruits and *Piper nigrum* seeds as effective fumigants for cereals against *T. castaneum*.^[5-9] The essential oil of *A. annua* has been shown to inhibit hatching of eggs and as a larvicide against *T. castaneum*.^[5] Powders of certain aromatic spices such as black pepper, cumin, coriander, and garlic have also been observed to control the *T. castaneum* infestation.^[10] The above methods require various extraction processes, fractionation of components, and mechanical application directly to the food material. Thus, there arises a need for such a material which could be easily available and could control infestation in stored food materials by indirect contact.

Recently, Weathers *et al.*^[11] have developed tablets from dry leaves of specific *A. annua* cultivar(s) that were highly effective in the treatment of acute and severe malaria. *A. annua* has been granted the generally recognized as safe status and therefore its leaves (≤ 30 g dry weight/day) can be safely consumed.^[12,13] It is reported that the 500 mg *Artemisia annua* Linn. dry leaf therapy (ALT) tablets, administered two per day for 5 days, cured the patients of complicated malaria, that had not responded to the World Health Organization recommended treatments.^[14] The chemical analysis of these tablets showed that these contain volatile and non-volatile secondary metabolites present in the leaves of *A. annua*, in quantities that survived the process of leaf drying.^[14,15] ALT tablets contained the following: Artemisinin (0.7–1%), other artemisinic compounds such as arteannuin B, artemisinic, and dihydroartemisinic acids; the coumarin scopoletin, many flavonoids such as artematin, casticin, circilineol, chrysoplenetin, chrysoplenol D, eupatorine, kaempferol, luteolin, myrcetin, and quercetin; chlorogenic acid; saponins; sulfated polysaccharides; and many terpenoid compounds, such as artemisia alcohol, artemisia ketone, borneol, camphene, camphor, caryophyllene, 1,8-cineole, germacrene-D, limonene, myrcene, nerolidol, α -pinene, phytol, sabinene, spathulenol, and α -terpineol.

In experimental animal studies, it was found that the blood of mice administered with dry leaves of *A. annua* contained

more than 40 times the artemisinin as compared to mice fed with pure artemisinin.^[16] Further, it was found that in the *Plasmodium chabaudi* infected mice, a single dose of *A. annua* leaves eliminated 5-fold more parasites than an equivalent dose of pure.^[17] The implication was that there was synergism between artemisinin and other secondary metabolites present in the leaves for curing of malaria in individuals administered with ALT tablets.^[18] It is expected that all biological conditions (ailments, infections, etc.) that respond curatively to artemisinin, flavonoid, and/or terpenoid components of *A. annua* should also respond to ALT tablets. It was already known that 1,8-cineole from *A. annua* is inhibitory to *T. castaneum*.^[6] Accordingly, in the present study, the activity of ALT tablets against *T. castaneum* was investigated.

P. nigrum L. (= *Piper aromaticum*; order Piperales; and family Piperaceae) drupes (fruits) are the source of black peppercorns. To obtain the peppercorns, the green drupes are dried as such or after light cooking in hot water. The average weight of a dry black peppercorn is 50 mg. The powdered peppercorns result in highly priced pungent spice, the black pepper, which is consumed as a spice, without any harmful effects to humans. The pepper powder contains oleoresins, alkaloids such as piperine, chavicine, piperic acid and piperidine, polysaccharides, flavonoids, and terpenoids, and among other substances.^[19-21] The alkaloid piperine is known to be a bioenhancer that increases the bioavailability of other compounds in animal systems.^[21,22] The main terpenoids that comprise the volatile component of pepper are levo-cineole, phellandrene, α - and β -pinene, limonene, sabinene, myrcene, 3-carene, borneol, linalool, carvone, caryophyllene, safrole, and myristicin.^[23,24] As, the essential oil of *P. nigrum* seeds and pepper powder had been shown to be inhibitory to *T. castaneum*; therefore, in the present study, it was investigated if addition of *Piper nigrum* seed powder (PSP) to ALT (ALT + PSP) tablets led to alteration in activity of ALT toward red flour beetles.

In the present study, three set of experiments were carried out. In the experiment no. 1, the ALT tablets of *A. annua* var. *Sanjeevani* and *Jeevan Raksha* were tested for their effect on the life cycle of *T. castaneum* under natural environmental conditions. In experiments no. 2 and 3, inhibitory effects of ALT and combination of ALT + PSP tablets of both varieties were quantified separately on adult insects and eggs of *T. castaneum* life cycle.

MATERIALS AND METHODS

Preparation of ALT Tablets (*A. annua* L. var. *Sanjeevani* and *Jeevan Raksha* Dry Leaf Powder)

Seeds of the *Sanjeevani* and *Jeevan Raksha* variety of *A. annua* were sown in a pot on December 14, 2016. Seedlings were transplanted to a field plot on March 1, 2017. Leaves

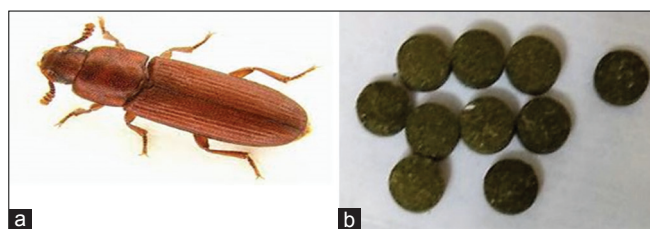


Figure 1: (a) *Tribolium castaneum* adult insect. (b) Tablets made of *Artemisia annua* var. *Sanjeevani* dry leaf and *Piper nigrum* seed

from the apical portions of the branches were harvested in the 1st week of May and dried indoors at 27–30°C temperature. The dried leaves were powdered separately and stored in glass bottles and retained at ambient room temperature. Tablets (each weighing 600 mg) of both the varieties were prepared a day before the start of the experiment by use of a compression machine. The tablets were compressed with the help of Proton Engineering's 16 station multi tooling machine using 12 mm biconcave punch.

Preparation of ALT + PSP Tablets (*A. annua* L. var. *Sanjeevani* and *Jeevan Raksha* dry Leaf Powder + *P. nigrum* L. Seed Powder)

A. annua dry leaf powders of both varieties were mixed separately with *P. nigrum* seed (peppercorn) powder in the ratio of 5:1. The mixture was used to prepare 600 mg ALT + PSP tablets [Figure 1b] using the same compression machine. The pepper seeds used were of the kind where drupes had been dried without cooking in hot water.

Experiment 1 (Insect Rearing)

Adult *T. castaneum* insects were collected from a food grain market in Delhi from a sack of wheat infested by the insect species. From the collected insects, 20 were released into an aluminum container having 2 kg of wheat flour. Wheat flour provides a conventional medium for experimentation on *T. castaneum*. The container was covered with its lid and placed in a storeroom. 2 months later, the adult beetles that became available were used in the experiment. An additional 1 kg of flour was added to the container to harvest adult insects for the subsequent requirement.

Experiment 1 (Toxicity Testing)

The experiment was carried out in 200 ml capacity glass jars. 20 g of wheat flour was added to each of 36 glass jars. One ALT tablet of var. *Sanjeevani* was placed on the surface of the flour in each of the 12 jars. Likewise, variety *Jeevan Raksha* tablets were kept on the surface of the flour of another 12 jars. 3rd set of 12 jars were devoid of ALT tablet. 11 adult *T. castaneum* insects were released into each of 36 jars. The mouth of jars was covered with a muslin cloth, tied with a thick cotton thread. The insects were released into the experimental jars on July 11, 2017 (in the hot and humid, rainy season) and the trays containing the randomly accommodated jars were kept in a storeroom in the dark. The experiment was terminated on August 23, 2017, when the number of dead and alive adult insects present in each of the jars was counted.

Experiments 2 and 3 (Insect Rearing)

Twenty plastic jars of 1 L capacity were used. 20 g wheat flour aliquots were poured into each jar. 10 *T. castaneum*

insects were released into each of the jars. The mouth of the jar was covered with muslin cloth and tied with thick cotton thread. The jars containing flour and insects were incubated in a growth chamber at 30°C, 65% R.H. and exposed to 12 h light and 12 h dark photoperiod. Adult insects were collected after 45 days of incubation.

Experiment 2 (Testing of Treatments for Adult Insect Mortality)

There were five treatments: Control, ALT tablet of var. *Sanjeevani*, ALT tablet of var. *Jeevan Raksha*, and ALT + PSP tablet of var. *Sanjeevani*, and var. *Jeevan Raksha*. For each treatment, a set of 10 glass jars of 200 ml capacity were used. To each jar, 20 g of wheat flour was added. One ALT tablet each of both varieties was placed on the floured surface in 1st and 2nd set of 10 jars. Likewise, a tablet each of both varieties of ALT + PSP was placed in 3rd and 4th set of 10 jars, whereas the remaining 10 jars served as control. 10 adult *T. castaneum* insects were released into each jar. The opening of each jar was covered with a muslin cloth, tightened with a rubber band. The experimental jars were incubated in a controlled environment chamber with 30°C temperature, 65% R.H. and 12 h light:12 h dark photoperiod, for 5 days. Dead insects were counted in each jar.

Experiment 3 (Testing of Treatments for the Inhibition of Insect Growth)

Insects were reared in as in section (e) above. Four (1 L capacity) jars were taken to which 200 g of wheat flour was added. 10 adult *T. castaneum* insects were added to each jar. The insect infested jars were incubated under ambient conditions of the environment controlled chamber as in (f) above. After 3 days, the contents (flour infested with *T. castaneum* eggs) of all the jars were pooled and sieved to remove the parental adult insects. To 50 glass jars each of 200 ml capacity, infested flour was added till leveled. The jars were divided into five lots of 10 each. The first set of 10 jars served as control. One ALT tablet each of var. *Sanjeevani* and *Jeevan Raksha* was placed on the surface of infested flour in a 2nd and 3rd set of 10 jars. Similarly, the infested flour in the 4th and 5th set of 10 jars was placed with a tablet each of both varieties of ALT + PSP treatment. The experimental jars were incubated at 30°C temperature, 65% R.H. and 12 h light:12 h dark photoperiod in a controlled environment chamber. After 47 days of incubation, the adult insect present in each of the 50 jars was counted.

Statistical Analysis

The statistical differences among different groups were tested by the one-way analysis of variance, using GraphPad Prism Software. $P < 0.05$ was considered statistically significant. The results of the three experiments are presented in Tables 1-4 and Figure 2.

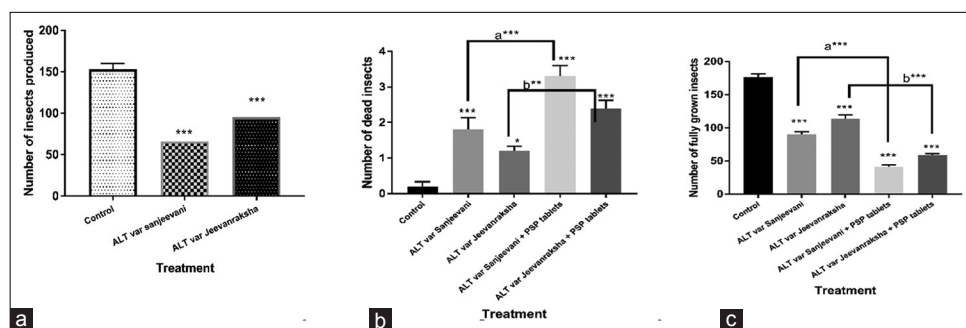


Figure 2: (a) Effect of *Artemisia annua* L. var. *Sanjeevani* and *Jeevan Raksha* dry leaf powder (ALT) tablets on the fecundity of *Tribolium castaneum*. Results were expressed as mean + Standard error of the mean (S.E.M). ($n = 12$) * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus control group. (b) Effect of ALT tablets and *Piper nigrum* L. seed powder (PSP) tablets on the survival of *T. castaneum* insects. Results were expressed as mean + S.E.M. ($n = 10$) * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus control group. (c) Effect of ALT tablets and ALT + PSP tablets on the growth of eggs, larvae, and pupae into adults of *T. castaneum*. Results were expressed as mean + S.E.M. ($n = 10$) * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus control group

Table 1: Effect of *A. annua* L. var. *Sanjeevani* and *Jeevan Raksha* dry leaf powder (ALT) tablets on the fecundity of *T. castaneum*

Number of insects produced after the treatment with	Jar 1	Jar 2	Jar 3	Jar 4	Jar 5	Jar 6	Jar 7	Jar 8	Jar 9	Jar 10	Jar 11	Jar 12	Total	Mean
Control	155	180	145	111	124	151	174	152	149	130	195	172	1838	153
ALT var. <i>Sanjeevani</i> tablet	96	100	56	45	13	91	74	109	12	94	31	74	795	66
ALT var. <i>Jeevan Raksha</i> tablet	105	122	75	84	67	122	120	98	42	117	98	95	1145	95

T. castaneum: *Tribolium castaneu*. ALT stands for *Artemisia annua* L. var. *Sanjeevani* and *Jeevan raksha* dry leaf powder tablets

Table 2: Effect of *A. annua* L. var. *Sanjeevani* and *Jeevan Raksha* dry leaf powder (ALT) tablets and *Piper nigrum* L. seed powder (PSP) tablets on the survival of *T. castaneum* insects

Number of dead insects after treatment with	Jar 1	Jar 2	Jar 3	Jar 4	Jar 5	Jar 6	Jar 7	Jar 8	Jar 9	Jar 10	Total	Mean
Control	0	0	1	0	0	0	1	0	0	0	2	0.2
ALT var. <i>Sanjeevani</i> tablet	1	1	2	3	2	1	4	1	2	1	18	1.8
ALT var. <i>Jeevan Raksha</i> tablet	1	1	2	1	1	1	1	1	2	1	12	1.2
ALT var. <i>Sanjeevani</i> +PSP tablet	2	3	5	3	4	4	3	3	2	4	33	3.3
ALT var. <i>Jeevan Raksha</i> +PSP tablet	2	2	4	2	2	2	3	2	2	3	24	2.4

ALT stands for *Artemisia annua* L. var. *Sanjeevani* and *Jeevan Raksha* dry leaf powder tablets. PSP stands for *Piper nigrum* L. seed powder tablets

RESULTS AND DISCUSSION

In north-west India's Indo-Gangetic plains area, the monsoon season starts from late June to early October with hot and humid conditions which are favorable for the infestation of stored-food materials by *T. castaneum*. The insect undergoes rapid and abundant growth during this period, leading to a

lot of spoilage of food materials. The experiment-1 of the present study was conducted to examine the effect of ALT tablets of var. *Sanjeevani* and *Jeevan Raksha* on the growth of insects on wheat flour under natural conditions in monsoon season. Insects to be used were reared on wheat flour. The experiment comprised three treatments replicated 12 times. The first set of 12 jars contained wheat flour only, the 2nd set

Table 3: Effect of ALT tablets and ALT+PSP tablets on the growth of eggs, larvae, and pupae into adults of *T. castaneum*

Number of fully grown insects after treatment with	Jan 1	Jan 2	Jan 3	Jan 4	Jan 5	Jan 6	Jan 7	Jan 8	Jan 9	Jan 10	Total	Mean
Control	155	189	180	165	150	175	193	162	208	182	1759	176
ALT var. <i>Sanjeevani</i> tablet	126	78	96	84	80	91	88	72	91	95	901	90
ALT var. <i>Jeevan Raksha</i> tablet	131	140	104	96	100	142	125	75	120	98	1131	113
ALT var. <i>Sanjeevani</i> +PSP tablet	31	36	40	48	40	35	65	37	38	40	410	41
ALT var. <i>Jeevan Raksha</i> +PSP tablet	65	57	52	58	67	52	74	50	62	51	588	59

ALT stands for *Artemisia annua* L. var. *Sanjeevani* and *Jeevan Raksha* dry leaf powder tablets. PSP stands for *Piper nigrum* L. seed powder tablets, *T. castaneum*: *Tribolium castaneum*s

of 12 jars contained wheat flour and one ALT tablet of var. *Sanjeevani* while the 3rd set contained wheat flour and one ALT tablet of var. *Jeevan Raksha*. 11 adult *T. castaneum* insects were released into each jar to infest the flour and allowed to breed their. After a period of incubation, when the insects were expected to complete a cycle of development, it was found that as compared to control jar, the progeny adults produce with about 57% and 38% less frequency in the jars containing ALT tablet of var. *Sanjeevani* and *Jeevan Raksha*, respectively. The result shows that ALT tablets inhibited the insect life cycle at all stages of development.

To investigate whether the presence of ALT tablet led to the death of adult *T. castaneum*, the adult insects were released over wheat flour in the presence and absence of ALT tablet. In experiment-2 an additional treatment of ALT + PSP tablet of both varieties was used to understand if the effect of ALT of var. *Sanjeevani* or *Jeevan Raksha* was modulated by PSP. Following the treatment, it was found that whereas 12% of the insects died in the presence of ALT var. *Jeevan Raksha* tablet, the mortality was higher at 18% in the presence of ALT var. *Sanjeevani* tablet. Likewise, after the treatment, it was found that whereas 24% of the insects died in the presence of ALT var. *Jeevan Raksha* + PSP tablet, the mortality was higher at 33% in the presence of ALT var. *Sanjeevani* + PSP tablet. The effect of ALT and PSP on the survival of *T. castaneum* adults was perhaps synergistically inhibitory.

In experiment-3, it was investigated if ALT and ALT + PSP combination had an inhibitory effect toward egg to adult developmental stages (including larval and pupal stages) of *T. castaneum*. The infested wheat flour was exposed separately to both varieties of ALT tablet, and ALT + PSP tablet during the insect's one life cycle period whereas the absence of both ALT and ALT + PSP tablets served as control. It was observed that the life cycle of *T. castaneum* was attenuated by var. *Jeevan Raksha* ALT and by its ALT + PSP tablets in such a way that progeny size was reduced

by these treatments as compared to control by 36% and 67%, respectively, whereas, var. *Sanjeevani* ALT and its ALT + PSP tablets reduced progeny size by 49% and 77%, respectively. Again, there was an indication of synergism between the inhibitory effects of ALT and PSP on life cycle developmental stages of *T. castaneum*. It was found that var. *Sanjeevani* ALT + PSP tablets were more effective than other tablets against growth and survival of *T. castaneum* insect.

CONCLUSION

It is already known that the whole essential oil distilled from *A. annua* shoots, its fractions; 1,8-cineole and limonene and the whole essential oil distilled from the seeds of *P. nigrum* (rich in limonene) repel the adult *T. castaneum* insects and are inhibitory to their various developmental stages, such as eggs, larvae, and/or pupae. Further, it is known that the *P. nigrum* seed powder coated seeds of wheat had been used against the insects. The present work shows that tablets made from dried leaves of *A. annua* plants, alone or in combination with *P. nigrum* seed powder were found to be inhibitory to adults as well as at various developmental stages of *T. castaneum* insect. The tablets were effective simply by their presence on the infested wheat flour surface. Thus, the secondary metabolites present in the dried leaves of *A. annua* and dry seed powder of *Piper nigrum* reached their target by evaporation cum-diffusion into the air and wheat flour present in muslin cloth enclosed glass jars. Therefore, it is possible to conclude that addition of ALT and/or ALT + PSP tablets into the outer packaging of the processed and stored food materials such as sacks or containers of cereals and legume grains/flours could provide effective protection against *T. castaneum*.

The use of ALT or ALT + PSP tablets would be highly cost-effective. The estimated cost of an ALT tablet is 5 paise (or cost of 1500 tablets = US \$ 1= Rupees 65). The ALT +

Table 4: Inhibitory effects of ALT tablets and ALT+Pepper (*P. nigrum*) seed powder (PSP) tablets on the red flour beetle (*T. castaneum*) that infests stored food products

Experiment number	Averaged parameter	Treatment					Critical difference at	
		Control	ALT var. Sanjeevani tablet	ALT var. Raksha tablet	ALT var. Jeevan Raksha+PSP tablet	ALT var. Jeevan Raksha+PSP tablet	5%	1%
1	Number of the adult insects produced	153	66	95	Not applicable	Not applicable	25.97	36.94
2	Number of mortality of adult insect	0.2	2	1	3	2	0.75	1.07
3	Number of fully grown insects	176	90	113	41	59	15.44	21.96

ALT stands for *Artemisia annua* L. var. Sanjeevani and Jeevan Raksha dry leaf powder tablets. PSP stands for *Piper nigrum* L. seed powder tablets

PSP tablets are estimated to cost at about 10 paise (or 7500 tablets for US \$ 1 = Rupees 65). In the household, to protect material such as 1 kg rice or split chickpeas into plastic or steel containers, 10 to 20 ALT + PSP tablets could be used that are costing less than Rupees 2 only. Trials of tablets on stored food materials are required to standardize the number of tablets to be used for different kinds of stored foods by their weight/volume and period of storage.

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