Biopesticide treated double layered bags: novel method of application of botanicals for *Sitophilus oryzae* L. management in stored maize

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Abstract

This study was conducted in order to evaluate the efficacy of the novel method of application of leaf powder of *Tinospora cordifolia* as water-based paste between the layers of double layered storage bags and acetonic leaf extracts on single layered bags against *Sitophilus oryzae* to protect stored maize for a period of five months. Untreated double and single layered bags were set as controls. After 5 months of storage, the weevil population builds up, percent grain damage, and percent weight loss was significantly lower in *T. cordifolia* treated double layered bags @ 2.0% w/w. However, untreated single layered bags recorded high population build up and maximum percent grain weight loss. The present work suggests that *T. cordifolia* treated double bags provide better protection to maize from *S. oryzae* infestation up to five months and can be readily used as an alternative to synthetic pesticides at the farm level.

KeyWords: *Stored Maize, Sitophilus oryzae, botanicals, Tinospora cordifolia, management*

Introduction

Maize (*Zea mays L.*) is one of the major cereal crop grown in India after rice and wheat. However, the yield of maize is low due to various biotic and abiotic factors. After harvest, maize is stored in traditional storage structures for food purpose and also for better market prices. Proper storage of grain is necessary to protect from insect pests, spoilage and also to increase keeping the quality. The damage to stored grain due to insects is 5-10% in temperate regions of the world and 10-30% in tropical countries (Nakakita 1998). Among insect pests, *Sitophilus oryzae* L. (Coleoptera: Driophthoridae) is the major pest of stored maize particularly in tropical regions causing severe losses because of its high reproductive potential. Both adults and larvae feed on whole grain resulting in extensive quantitative and qualitative losses of grain. The infestation of the weevil causes 53.30% damage to maize grain and 14% loss in weight over four months storage period (Lakshmi Soujanya et al.2013). Mixing of neem leaves (2-5%) with grain, combining neem leaf paste with the mud which is used for making earthen bins; overnight soaking of gunny bags in boiled neem leaf extract (2-10%) (Sharma 2016) and keeping of fresh Pongamia glabra leaves as layers in between the gunny bags (Kumar et al. 2015) were some of the traditional methods practiced in India for the management of storage pests. Kiruba et al. (2008) reported that leaves of *Azadirachta indica* and *Vitex negundo* effectively suppress the population of *Sitophilus oryzae*, *Tribolium castaneum* and *Corcyra cephalonica* in stored maize. Even though synthetic insecticides are effective for the control of storage pests, their repeated use leads to the development of resistance, residue development and environmental pollution (Dubey et al. 2007). Further, they are quite expensive to smallholder farmer situation. Moreover, the use of methyl bromide, an effective fumigant has been banned in India due to its ozone-depleting nature as per International agreement of Montreal protocol (Philips and Throne 2010). With limitations on the use of synthetic insecticides, there is an urgent need for safer alternatives particularly from plant sources for the protection of maize against storage pest infestation.

Plant-derived materials are readily biodegradable, less likely to contaminate the environment, less toxic to mammals; contains a rich source of bioactive compounds (Quin et al. 2010). *Tinospora cordifolia* (Figure 1) commonly named as "Guduchi" belongs to Menispermaceae family is a genetically diverse, large, deciduous climbing shrub with greenish yellow typical flowers. Phytochemical analysis of *T. cordifolia* indicated the presence of active components such as alkaloids, flavonoids, steroids, diterpenoid lactones, aliphatics, and glycosides. This plant captures great attention due to its medicinal properties such as antidiabetic, anti-allergic, anti-stress, anti-leprotic, anti-

Figure 1. *Tinospora cordifolia*

malarial, activities (Saha & Gosh 2012). Kim et al. (2012) reported that the present market share of biopesticides including botanical and microbial pesticides is less than 2.2% of global pesticide market. This might be due to adverse effects such as undesirable odor, the and color change of commodity that are associated with botanical pesticides when applied directly to the grain. Keeping in view of the above aspects, a new method of application of botanicals i.e. *T. cordifolia* treated double layered bags is investigated in the present study to protect stored maize against *S. oryzae*.

Materials and methods

The experiment was conducted at Winter Nursery Centre, Hyderabad, Telangana. The culture of *S. oryzae* was maintained in the laboratory on whole maize grain (DHM 117) in plastic containers of 1l capacity at 26 ± 2 oC and relative humidity of 65 ± 5%.

Preparation of plant material

Fresh leaves of *T. cordifolia* were collected locally, shade dried for five days and ground in a grinder to a fine powder. Different concentrations of leaf paste 0.5% w/w, 1.0%w/w, 1.5%w/w, 2% w/w were prepared by taking 10, 20, 30 and 40 g plant powder, respectively. The plant powder was mixed with 10 g of starch per 100 ml of water to make a paste and was spread all over on the outer surface of the bags. The bags were kept to dry in a room for 24 hrs. 10 g of plant powder was dissolved in 100ml of acetone and kept for 24 hours. Later, the solution was filtered through Whatmann No 1 filter paper and condensed by evaporation of the solvent to 10 ml. From this stock solution, 60 ml of 2.0 % v/v spray fluid was prepared and sprayed on single layered gunny bags.

Experimental Procedure

The storage material used was gunny bag of 40 cms × 32 cms size. The treated bags were filled with 2 kg maize grain and then inserted into a second bag of the same size. Ten male and ten female adults of rice weevil were introduced into each experimental bag and tied with a rope tightly. The untreated double and single layered bags were constructed in the same way but were not treated with plant material. The experiment was replicated thrice with seven treatments by adopting completely randomized design. Double layered gunny bag treated with *T. cordifolia* leaf paste@ 0.5% (T1), Double layered gunny bag treated with *T. cordifolia* leaf paste@ 1.0% (T2), Double layered gunny bag treated with *T. cordifolia* leaf paste@ 1.5% (T3), Double layered gunny bag treated with *T. cordifolia* leaf paste @ 2.0% (T4), Single layered gunny bag treated with acetonic leaf extract of *T. cordifolia* @ 2.0% (T5) , Untreated double layered bag (T6), Untreated single layered bag (T7). All these bags were kept in the storeroom to test their level of protection from weevil population and grain weight loss. The experimental bags were sampled every four weeks for five months to assess the number of progeny present, the number of grains damaged and the amount of grain weight loss that had occurred due to weevil feeding was calculated after 90 and 150 days of treatment by following the formula given by Gwinner et al. (1996).

Percent weight loss =
[(Wu × Nd) – (Wd × Nu)]/ [Wu × (Nd + Nu)] × 100

Wu = weight of undamaged grains

Wd = weight of damaged grains

Nu = number of undamaged grains

Nd = number of damaged grains

Percent Grain damage = w(Number of damaged grains/ Total number of grains) × 100

Statistical Analysis

The data collected were subjected to Analysis of variance (ANOVA) and was performed with SAS 9.3 version by using general linear model (GLM). Data on percent grain damage, weight loss was transformed with angular function to correct for heterogeneity of treatment variances (Gomez and Gomez, 1984) before being subjected to ANOVA. The transformed means were separated by Duncan's Multiple Range Test at 5% level of probability.

Table 1: Per cent grain damage and weight loss by Sitophilus oryzae to maize grain stored at various

Treatments	Per cent Grain damage		Per cent Weight loss	
	90 DAS	150 DAS	90 DAS	150 DAS
Tinospora cordifolia leaf paste @0.5%	5.0 ± 1.7C	9.33 ±0.6B	1.04 ± 0.66AB	1.71 ± 0.92B
T. cordifolia leaf paste @1.0%	4.33± 0.88C	6.66 ± 0.3B	0.89 ± 0.89B	1.50 ± 1.17B
T. cordifolia leaf paste @1.5%	3.66 ± 0.3C	5.6 ± 1.3B	0.81 ± 1.54B	0.96 ± 1.32B
T. cordifolia leaf paste @2%	3.00 ±0.5C	5.3 ± 0.6B	0.87 ± 1.88B	0.92 ± 1.39B
Acetonic leaf extract of *T. cordifolia* @ 2.0%	13.00 ± 0.5B	24 ±2.0A	1.82 ± 1.43AB	3.89 ± 0.52A
Untreated double bag	15.33 ± 0.3B	25.3 ± 1.6A	2.39 ± 1.73AB	4.50 ± 0.72A
Untreated single bag	22.00 ± 1.1A	28 ± 2.0A	3.17 ± 0.98A	5.41 ± 0.58A

Means within a column followed by different letters are significantly different (Duncans Multiple Range Test p = 0.05) DAS: Days after storage

Results

Effect of *T. cordifolia* treated gunny bags on the adult emergence of *S. oryzae* infesting stored maize

Results pertaining to the mean number of F1 progeny emergence of *S. oryzae* on stored maize after 30, 60, 90, 120 and 150 days of treatment were presented in *Figure 2*. It was observed that the number of adults emerged vary with the treatments. Throughout the study, treated double layered bags with leaf paste of *T. cordifolia* significantly reduced the adult emergence. At 30 days after treatment, there is no significant difference in F1 progeny emergence among all the treatments. The number of adult emergence was lowest in double layered bags treated with leaf paste of *T. cordifolia* (from 0.5 to 2% w/w) followed by single layered bags treated with acetonic leaf extract of *T. cordifolia* @ 2% v/v at 60 days after treatment. On the other hand, untreated double and single layered bags recorded a maximum number of weevil emergence. More or less similar trend of F1 progeny emergence was observed after 90, 120 and 150 days after treatment. However, weevil emergence decreased among all the treatments after 150 days of treatment. The over all mean number of adults emerged in double layered bags treated with leaf paste of *T. cordifolia* @ 2% w/w recorded lowest F1 population of 24.7 followed by 25.7, 25.8, 28.1 with 1.5%, 1.0% and 0.5% w/w leaf paste of T. cordifolia, respectively while acetonic leaf extract of *T. cordifolia* @ 2% v/v recorded 86.2 weevil population. Maximum weevil emergence was observed in untreated double and single layered bags (97.2, 129.7), respectively and they were significantly different compared to all the other treatments.

Effect of *T. cordifolia* treated gunny bags on the per cent grain damage and weight loss of maize kernels

Table 1 shows the percent grain damage and weight loss of maize kernels across different treatments over five months storage period. The percent grain damage was significantly high in maize stored in untreated double (15.33, 25.30) and single layered bags (22.0, 28.0) after 90 and 150 days of storage, respectively. The minimum damage of 3.0% to 5.3% was observed in double layered gunny bag treated with *T. cordifolia* leaf paste @2%w/w after 90 and 150 days of storage, respectively. At 90 days after storage, double layered

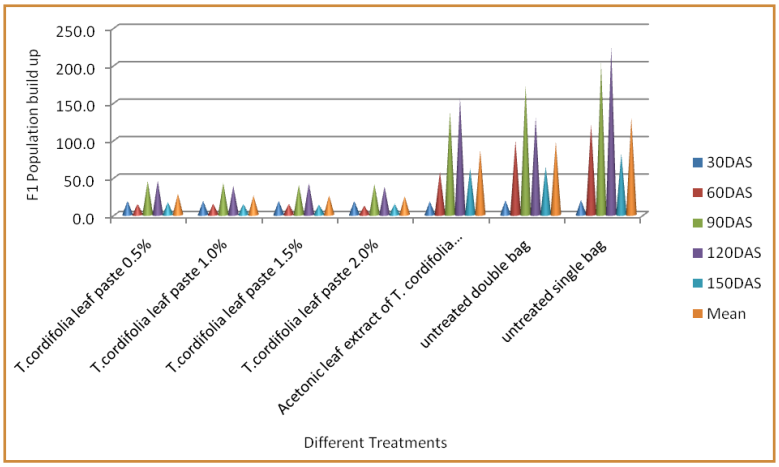

Figure 2. F1 population build up of *S. oryzae* in maize grain stored at various treatments

gunny bags treated with *T. cordifolia* leaf paste @ 1.5%, 1.0%, 0.5% showed percent damage of 3.66, 4.30, 5.0 while it was 5.60%, 6.60%, 9.33% after 150 days of storage, respectively. Single layered bag treated with acetonic leaf extract of *T. cordifolia* @ 2.0%v/v recorded 13.0 and 24.0 % grain damage after 90 and 150 days of storage.

Similar to percent grain damage, the percent weight loss produced as a result of weevil feeding activity was significantly higher in untreated double (2.39, 4.50) and single (3.17, 5.41) layered bags after 90 and 150 days of storage, respectively. The maximum protection against *S. oryzae* was observed in double layered gunny bag treated with *T. cordifolia* leaf paste @2%w/w which recorded minimum percent weight loss of 0.87, 0.92 after 90 and 150 days of storage. Double layered gunny bags treated with 1.5%,1.0%, 0.5% *T. cordifolia* leaf paste showed 0.81, 0.89 and 1.04% weight loss after 90 days of storage while it was observed as 0.96%, 1.5% and 1.7% after 150 days of storage while the acetonic leaf extract of *T. cordifolia* @ 5% recorded 1.82%, 3.89% after 90 and 150 days of storage, respectively.

Discussion

Double layered gunny bag treated with leaf paste of *T. cordifolia* significantly reduced the emergence of F1 progeny and percent grain weight loss. This is the first report on the bioactivity of *T. cordifolia* against *S. oryzae*. The findings of this research are in conformity with several workers who studied the efficacy of botanicals against *S. oryzae* in stored maize. Similar study was made by Utono et al. (2014) who reported that combination of *Ocimum basilicum* (Sweet basil) and *Cymbopogon nardus* (Lemongrass) powdered dried leaves was found more repellent to *Tribolium castaneum*, when applied as a water-based paste between the layers of double storage-bags at a dose of 1% w/w (plant powder/grain) than untreated double bags. Further, Ogungbite et al. (2014) reported that jute bags treated with Eugenia aromatica had the greatest mortality effect on the *Rhyzopertha dominica* within 48 hours of exposure and all the treated bags were found to reduce or prevent the weight loss and damage of the stored wheat after 42 days of storage. Earlier Jayaseelan et al. (2011) reported larvicidal activity of synthesized silver nanoparticles using an aqueous leaf extract of *T. cordifolia* against mosquitoes.

Plants belonging to Menispermaceae family contains L-tyrosine derived alkaloids which are responsible for repellent activity against insect pests in various studies (Feng et al. 2012; Elanchezhiyan et al. 2015). In view of significant protection found from leaf powder of T.cordifolia against maize pest, a detailed literature search has been made to understand its secondary metabolic composition. Few classes of compounds such as flavonoids- saponarin (Sengupta et al. 2009), steroids- β- sitosterol, ecdysterone (Dixit & Khosla 1971), diterpenoids such as columbine (Sarma et al. 1998) were earlier reported from the leaves of *T. cordifolia (Figure 3)*. The presence of active constituents such as terpenoids might be responsible for the significant protection of stored maize against *S. oryzae*. Interestingly, flavonoids, steroids (Auamcharoen et al. 2009) terpenoids (Kouninki et al. 2007) are reported to exhibit insecticidal activities against Sitophilus sp. In another study, Gutierrez (2016) reported that *Tinospora rumphii* powder containing alkaloids, flavonoids, steroids showed significant insecticidal activity against *S. zeamais*. Similarly, Gomah (2014) demonstrated toxic and antifeedant activities of prenylated flavonoids isolated from *Tephrosia apollinea* L. against *S. oryzae*. Further, it was reported that flavonoids in plants act as antioxidants, insecticides, antimicrobials, and repellents (Pieta 2000). In addition, it is also revealed that steroids take part in a defensive function by disrupting the insect's molting cycle when ingested by insect herbivores (Hopkins and Huner 2009). Further work on the screening of plant extracts, their fractions, identification, and isolation of bioactive chemical constituents from the leaf powder of *T. cordifolia* is in progress in our laboratory.

The paste of dried leaf material of *T. cordifolia*, when applied between the two layers of bags, formed concentrate barrier in which the insect multiplication was decreased. As the experimental bags were kept in the storeroom, there will be a chance of infestation

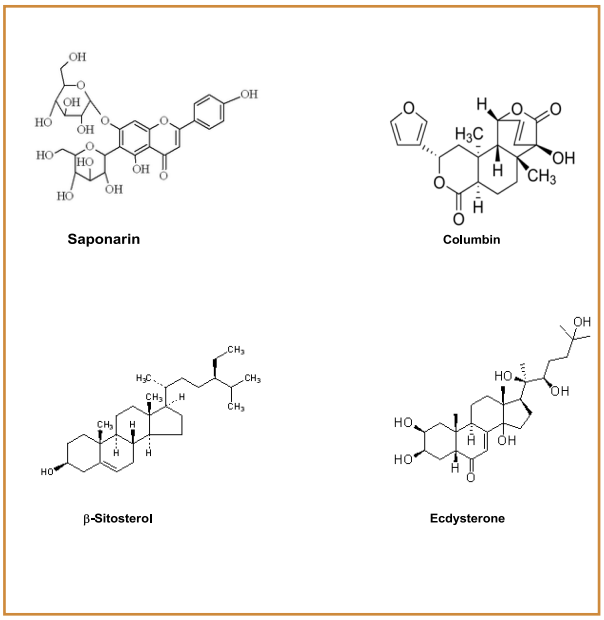

Figure 3. Secondary metabolites present in leaf of Tinospora cordifolia

of weevils to stored maize from the exterior place. However, entry of weevils from outside was prevented due to the repellent activity of leaf material. Moreover, it would be more difficult for weevils to penetrate into double-layered bags. Also, the double-layered bags do not allow air to flow in and maintained the moisture content of grain. Therefore, slow rate of increase in infestation of weevils was observed in treated double bags which may be enough to maintain a low level of infestation for a storage period of five months. Although there was a notable increase in progeny emergence, grain damage and weight loss of grain across all the treatments throughout the storage period, grain stored in the treated double-layered bag was better protected against *S. oryzae* when compared to the untreated single layered bag. Latha and Naganagoud (2015) reported that sweet flag rhizome treated sorghum seeds packed in a steel container, recorded lowest seed damage percentage (32.00%), number of live *S. oryzae* adults (5.11) after nine months of treatment. It was reported that surface treatment of gunny bags with the sweet flag at 5 percent caused the highest mortality of 96.65% and 97.30% with least live adult population of *S. oryzae* of 0.00% and 11.00% at 30 and 90 days after treatment, respectively (Biradar et al. 2016).

Untreated single bags comparatively recorded a maximum number of weevils due to insect penetration from outside and single layer support ventilation which results in an increase of moisture content of grain (Ali et al. 2009). As a result, a congenial environment has been created for the multiplication of insects. In case of untreated double bags, weevil population and weight loss were less compared to single layered gunny bags as weevils were unable to enter into double layered bags from outside. However, in all the treatments, there was a sudden decrease in insect number after four months which might be due to very high temperatures prevailing in the month of April.

The results of the present study indicate that double bagging method treated with leaf paste of *T. cordifolia* is cost-effective to smallholder farmers due to its ease of availability in nature and can keep grain free from pest damage for five months. In this method, there is no direct contact of grain with the botanical and is therefore superior to the farmers existing method of mixing grains with repellent plant materials. Based on the results, potential insecticides can be developed from *T. cordifolia* for the management of *S. oryzae* in stored maize.

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Disclosure Statement

No potential conflict of interest was reported by the authors.

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