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Effectiveness of bio-insecticide incorporated storage bag for safe storage of legumes

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ABSTRACT

India loses 2–6% of total produce due to lack of improper farm storage facilities. A potential novel method of incorporating the bioinsecticide (azadirachtin 0.15%) into the storage bag was evaluated for its effectiveness in insect control for safe pulse storage at Division of Agricultural Engineering, ICAR-Indian Agricultural Research Institute, New Delhi during 2021. Azadirachtin (2.41%) was incorporated into jute and woven polypropylene bag. The configuration with both sides treated jute and WPP bag produced mortality of 93 and 100% respectively against *Callosobruchus maculatus* adults. Both the treated bags produced mortality (>75%) within 48 h indicating the efficacy of selected bioinsecticide to contain infestation. The contact toxicity was dependent on porosity and *p*H of the storage bag material along with exposure time. Incorporation of the bioinsecticide saved more than 30% loss of weight in Bengal gram stored for 6 months. The storage quality of legumes, accessed as percentage of grains with holes and percentage of grains with eggs after storage duration of six months was within the acceptable values. The practice saved a value of ₹3242/t on stored Bengal gram in Jute bag and ₹206 in WPP bag respectively. Thus incorporation of azadirachtin could be potential tool for effective post-harvest storage of legumes in a sustainable manner.

Keywords: Azadirachtin, Bioinsecticide, Jute, Legumes, Storage, Woven polypropylene

Post-harvest losses of cereal, pulses and oilseeds due to insect pest are predominant constraint to food security in the developing nations. Along with consuming the grains, the insects contaminate the grain by their excreta, webbings and body fragments rendering them unfit for consumption. The post-harvest losses during storage have been quantified to be more than 1.67% in chickpea and 1.27% in green gram. In India, over 99% of the government procured grain is stored using bags in state owned warehouses. These bags are susceptible to high degree of infestation ranging to as high as 60%. Growing awareness of hazards caused by synthetic insecticides like genetic resistance, phototoxicity with high cost and other environmental concerns has fuelled researches on bioinsecticides for insect pest management in storage.

The bioinsecticides are cheap, easily available, target specific along with being safe to environment and humans. Incorporation of insecticides in storage bags is an emerging methodology for management of storage insects. Insecticides such as delthamethrin have been incorporated and found effective against several storage insects such as *T. granarium* and *C. chinensis* (Paudyal *et al.* 2017). Often the insecticide is directly applied on the packaging storage bag material (Scheff *et al.* 2016). It is evident that the adult stages of common storage insects have ability to fly and hence contact toxicity could be a tool for effective management against them. Bio extracts of neem, lantana camara, sesame, eucalyptus, safflower and many other plants have been reported to produce desirable efficacy against common storage insects of pulses (Gaydhane *et al*. 2021). The present study aimed at evaluating the effectiveness of bioinsecticide incorporated jute and polypropylene bag for post-harvest storage of legumes.

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MATERIALS AND METHODS

The experiments were conducted at Division of Agricultural Engineering and Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi during 2021. Insect culture of *Callosobruchus maculatus* was reared in kilner jar at 65% RH and 30°C.

Azadirachtin 0.15% (Neem Baan) was used as bioinsecticide for incorporation in the storage bags. Neem based extract was selected because of its easy availability, simple processing with negligible ill effects on environment and humans. Direct usage of Neem could lead to development of foul sulphurous odour so, incorporation of azadirachtin 0.15% in storage bag was considered. The bioinsectcides was incorporated in jute and woven polypropylene bag. Virgin jute bag (94 cm × 57 cm, warp 10 lb/spy, wept 21 lb/spy) and woven polypropylene food grade storage bag (mesh density 16 × 16/inch^2, 100% virgin polypropylene) was incorporation with bioinsecticide.

Selection of dosage: Based on the preliminary finding studies, five different doses were selected between 1–10% and 3 ml solution was sprayed uniformly on filter paper using glass atomiser (10 ml). Bioassay experiment using 10 adult insects of *C. maculatus* was conducted to find the contact toxicity of bio insecticides at various dosages. Based on the mortality values obtained, LC_{50} was calculated by log dose probit mortality regression analysis.

Contact toxicity bioassay: The experiment was conducted in RCBD with 3 replication and equal number of sub replicates. Circular strips of 9 cm diameter corresponding to base of petri dish were cut from fabric of storage bags. The strips were sprayed with 3 ml aqueous solution of azadirachtin 0.15% using hand held glass atomiser. The strips were affixed to the bottom of petri dish and the walls were coated with tetraflouroethylene (60%) to prevent escape of insects. Fixed population of adults (10) *C. maculatus* were released in the petri dish and their mortality was examined after 1, 24, 48 and 72 hours after exposure. The non-responsive insects on protruding acting by a probe were marked as dead.

Data analysis: Chi square test was used for determination of goodness of fit. POLO (probit or logit) software tool was used for log dose probit analysis. The mortality values obtained after bioassay were transformed through arc sine transformation and analysed using SPSS Inc. version 17.0. Variable exposure time was the repeated factor and mortality of insects was the response variable. Sprayed surface and type of storage bag material were the main effect. Tukey's HSD test (P=0.05) was used for separation of means.

Effectiveness of the bioinsecticide incorporated storage bags: Mini bags (21 cm × 16 cm) were prepared using the fabric cut from the storage bags of jute and woven polypropylene. The fabrics were treated with selected dosage of bioinsecticides and left for 24 h in controlled temperature and relative humidity conditions (30°C and 75% RH). The bags were filled with 500 g diet of green gram variety (Pusa Vishal). Twenty adults of *C. maculatus* were released in the bag and bags were sewn using hand sewing machine (Usha electric power sewing machine motor electric sewing machine, sewing speed 6000 SPM). The bags were transferred to the acrylic boxes (28 cm, Ø 15 cm) whose walls were treated with tetraflouroethylene. The boxes were placed in controlled temperature and RH conditions (28°C and 65% RH). The mortality of insects was examined after 12 h, 1, 2 and 7 days and the experiments were replicated thrice. The mortality of insects corresponds to the effectiveness of the treated bags to contain infestation.

Comparative evaluation: Bioinsecticides incorporated jute and woven polypropylene storage bags were compared with untreated bags for storage of Bengal gram. The grains were stored in three replicates of each treatment for prolonged duration of six months. The 500 kg of Bengal gram (*Cicer arietinum*) variety Swetha (ICCV-2) was stored over a prolonged duration of 6 months from March to September. 30 kg batches of the pulse were stored in each variant of bag with three replications. The storage interval of six months was selected because it represents a typical storage time used by the farmers until the grain is consumed or sold. It also provided enough time for insects (predominantly the pulse beetle) to reproduce 4–5 cycles which would have been sufficient for causing severe damage to the stored grain. The bags were kept on 0.5 m high dunnage in vertical positions maintaining a suitable distance to reduce chances of cross contamination from one bag to the other. The procured grain has minimal initial infestation.

The infestation level in the storage Bengal gram was assessed at one month interval along with the beginning of the experiment and after six months. Twelve, 200 g samples (n=12) were randomly collected from each treatment (three samples per replicate). Each 200 g sample was sieved to separate and counted live adults of each insect species. Three sub-samples of 100 grains were randomly taken from each of the three 200 g samples, resulting in 900 grains per replicate (n=36 per treatment). These 100 grain sub-samples were used to assess insect damage grain with holes and weight loss. The percentage of insect-damaged grain and the weight losses was calculated as:

$$\text{Per cent (grain with holes)} = (\text{Number of damaged grain with holes}) \times 100 / (\text{Total grain count})$$

$$\text{Per cent weight loss} = (DWo - DWt) \times 100 / DWo$$

where DWo, the dry weight at the beginning; DWt, dry weight after storage interval (t).

Economics: Return on storage primarily depends on commodity price at harvest, quantity of grain to be stored, length of storage period, price seasonality (increase or decrease) across the storage period, cost of storage technology, damage weight loss during storage, percentage of damaged grains, and price discount for damaged grains. The economic analysis of the developed storage bag mainly involves the assessment of the value generated by saving the grain from deterioration and the profit earned by storing the produce for the particular time period. The difference of the market value (price) of Bengal gram at several selling times was considered as the opportunity cost of each storage method and incorporated into the selling price for gross margin calculations (Alemu *et al.* 2021). The cost that varied with each storage technique was included in estimation of net benefits of the storage method.

RESULTS AND DISCUSSION

Selection of applicable dose of azadirachtin 0.15% for incorporating the storage bags was conducted by evaluating its efficacy against *C. maculatus* adult. The selected bioinsecticide was toxic against *C. maculatus* at all the five selected dosage within a day of exposure duration. However, the desired mortality (>75%) was evidently observed at dosage greater than 50000 ppm (5%) (Fig 1).

LC_{50} values for the selected bioinsecticide were calculated to be 24000 ppm (2.4%). As a result of incorporation, active ingredient of 1.20 mg a.i./cm^2 was produced over the treated surface. This bio-insecticidal application dose was used for incorporating azadirachtin 0.15% into the storage bags.

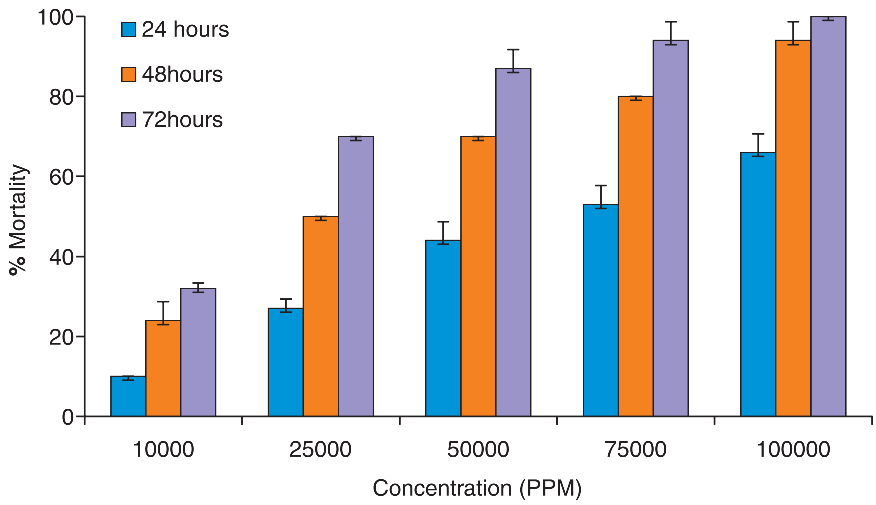

Fig 1 Mortality of *Callosobruchus maculatus* at different dosage of azadirachtin 0.15%.

In the bioassay experiment for evaluating the contact toxicity on different storage bag materials treated with the selected bioinsecticides, the main effect of sprayed surface produced significant difference (P>0.05) on the dependent mortality value. Different storage bags (Jute and WPP) and their interaction effect (sprayed surface × storage bag) however showed no significant effect on the mortality of exposed insect (*C. maculatus*). Within the exposure interval the exposure interval × sprayed surface and exposure time × type of storage bag material both produced significant effect on mortality evidently highlights the dependency of mortality on the exposure duration. The efficacy of Neem based extracts have also been reported by Tulashie *et al.* (2021) against fall army worm, Chaudhary *et al*. (2017) against *Sitophilus Oryzae* and *Callosobruchus maculatus*. The contact toxicity of bioinsecticide on storage bag fabric (Jute and WPP) depends on its active ingredient concentration, porosity of fabric and its surface *p*H (Kavallieratos *et al.* 2017, Arthur *et al.* 2018).

In both side applications, the active ingredient produced was higher for the treated bags which resulted in higher mortality of *C. maculatus*. WPP bag fabric has lower porosity compared to jute bags hence the loss of active ingredient from the surface to substrata was low. In addition to porosity, the alkaline nature of treated jute bag produced hydrolysis and degradation of insecticides diminishing its efficacy and hence reduced mortality effects have been reported (Arthur *et al.* 2018). Exposure on the non-treated side of the jute fabric produced higher mortality than WPP bag because the porous nature of fabric allowed higher amount of bioinsecticides to seep through and produce toxicity on the non-treated side. *C. maculatus* adults are agile (mobile) in nature and hence contact toxicity is a potential tool for management of such beetles. In case of WPP mini bags, the lack of oxygen and enriched carbon dioxide environment (hermetic) produced by respiration of grains and insects and low gas permeability of bag produced control of infestation comparable to treated jute bags. The combined effect of toxicity in treated WPP bags and hermetic effect produced highest degree of control from insect infestation. Similarly, the loss of weight was lowest for bioinsecticide incorporated WPP bags as an outcome of lowest infestation due to insects. Similar protection effect has been studied by Mobolade *et al*. (2019). The mortality value on first hour of exposure varied from 13.34–23.34% in jute bags and 6.66–20.00% in WPP bags. At the end of 72 h exposure interval, highest mortality in the treated jute bag was for the dual surface treated bag (93%), the same configuration achieved complete mortality of the insects in case of WPP bags. Exposure of insects on the non-treated side produced lower mortality in case of WPP but was equally effective in jute bag fabric material. The treatment configuration of both sides treated produced lowest LT_{50} value for both the storage bags (Table 1).

For evaluating the effectiveness to contain the infestation within the bag, treated mini bags were compared with untreated mini bags. The treated bags produced significantly higher mortality in the insects inside the bag

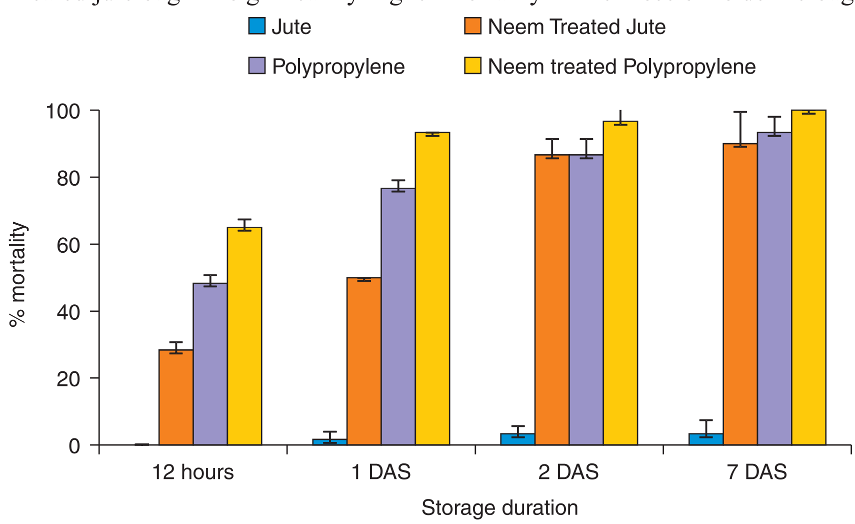

Fig 2 Mortality of *Callosobruchus maculatus* stored in various mini storage bags of greengram.

Table 1 Contact toxicity (mean immediate ± SE mortality (%)) of azadirachtin 0.15% against adults of *C. maculatus* exposed on different storage bag fabrics

Storage Bag Material	Treatment	1 hour	24 hours	48 hours	72 hours	F	P	Regression equation (R^2, LT_{50})
Jute	One surface is treated & insect exposed to the treated surface	23.34 ± 3.34 ABd	33.34 ± 3.34 Bc	56.00 ± 3.34 CDb	73.34 ± 3.34 BCa	46.0	<0.01	0.60 x + 4.17 (0.71, 22.55)
	Both surfaces are treated	23.34 ± 3.34 ABd	43.34 ± 6.67 ABc	80.00 ± 5.77 ABCb	93.34 ± 3.34 Aa	41.7	<0.01	1.03 x + 4.11 (0.74, 7.23)
	One surface is treated & insect exposed onto untreated surface	13.34 ± 3.34 BCd	26.67 ± 3.34 Bc	53.34 ± 3.34 Db	73.34 ± 3.34 BCa	65.0	<0.01	0.78 x + 3.79 (0.76, 33.93)
Woven Polypropylene	One surface is treated & insect exposed to the treated surface	20.00 ± 0.00 ABd	40.00 ± 5.77 Bc	66.67 ± 3.34 BCDb	86.67 ± 3.34 Aba	61.8	<0.01	0.89 x + 4.02 (0.77, 12.45)
	Both surfaces are treated	20.00 ± 0.00 ABd	66.67 ± 3.34 Ac	93.34 ± 3.34 Ab	100.00 ± 0.00 Aa	237	<0.01	1.75 x + 3.90 (0.78, 4.21)
	One surface is treated & insect exposed onto untreated surface	6.66 ± 3.34 Cd	26.66 ± 6.67 Bc	50.00 ± 10.00 Db	66.67 ± 3.34 Ca	16.6	<0.01	0.93 x + 3.43 (0.90, 46.63)

Within each row, means followed by the same lowercase letter are not significantly different. Within each column, means followed by the same uppercase letter are not significantly different, where no letters exist, no significant differences were recorded.

for both jute and WPP bags. However, the mortality of insects in WPP bags was similar to treated jute bags (Fig 2). The toxic effect incorporated in the fabric was effective in containing infestation.

Comparative analysis of storage bags for prolonged duration of 6 months was conducted by storing Bengal gram cultivar Swetha. Loss of weight in Bengal gram as a result of insect infestation reduced significantly in bioinsecticide incorporated storage bags. The reported loss of weight was 33.04% in jute bags which reduced to 1.88% after treatment. In WPP, the loss of weight was 25.12% and less than 1% for untreated and treated bags respectively. The insect population declined in both the azadirachtin 0.15% treated bags (Jute and WPP). However, treated WPP bags showed superior results in controlling infestation of insects. Moisture absorbance of the jute bags aided to growth of insects and hence forth high damage. Interestingly, the number of insects in WPP bag increased from the initial number contrary to the mini bags of WPP. This is due to the larger size of 50 kg storage WPP bags providing sufficient oxygen for insects to grow and the larger surface area of bag resulted in higher gas permeability compared to mini bags of WPP.

Similar results were observed for per cent grain with holes, which spiked to as high as 52% from initial value of 24.37% in case of untreated jute bag. The percentage of grain with holes remained almost same in both the treated bags. Percentage of grains with eggs on them decreased from its initial value in case of treated jute and WPP. It spiked to as high as 74% from 21% in untreated jute bag (Table 2). The reduction in both the parameters of deterioration in quality highlight the protective effect produced by treated of azadirachtin 0.15% on the storage bags.

Loss of grain due to damage by insects over the storage period and change of price at start and end of storage period were the primary criteria for studying the economics of storage.

The difference in weight loss of the grain sample from the treated and non-treated bags was considered as grain saved as a result of bioinsecticide incorporation in the storage bag. In 50 kg storage bag, predominantly used for rural storage, incorporation of azadirachtin 0.15% saved value of ₹162 in jute bags and ₹60.3 for WPP bags. The value saved per tonne of Bengal gram stored was ₹3242 and ₹1206 from the treated and non-treated bags respectively. The cost of treating the bag was minimal and hence was neglected. Other cost incurring on monitoring, inspection and handling were same for both non-treated and bioinsecticide incorporated bags. It is well known that grain saved is vital than grain produced. The higher value was generated in treated jute bags compared to WPP as degree of protection developed in high susceptible jute bag was more compared to WPP bags. The value generated can also aid in regulating the demand supply chain and check the rampant inflation of prices for legumes.

Incorporation of azadirachtin (Neem Baan 0.15%) in the grain storage bag is a potential alternative for post-harvest management of legumes. Apart from saving the loss of grain due to insect infestation, as the noble method of application reduces the chances of transfer of toxicity to grain and eliminates the foul smell of grain observed in case of direct treatment. Both jute and WPP bags treated

Table 2 Storage quality parameters in 50 kg bags of stored Bengal gram over 6 months

	N	Adult insect/ 500 g grain sample	n	% grain with holes	%grain with eggs	Initial Weight (g) (100 kernel)	Final weight (g) (100 kernel)
Initial	36	22 ± 1.63	108	24.33 ± 0.94	21.66 ± 2.35	-	-
Jute Bag	9	38 ± 3.74	27	52.66 ±3.29	74.33 ± 4.92	32.45 ±0.07	21.74± 0.05
Neem treated jute bag	9	7±1.41	27	25.33 ± 2.05	12.33 ±2.05	29.78± 0.05	29.22±0.06
Woven polypropylene	9	31± 1.63	27	33.33 ± 2.35	56±5.65	32.08± 0.09	24.02± 0.09
Treated woven polypropylene	9	5.33 ± 2.05	27	25 ±1.63	9.33 ± 0.94	33.08 ±0.10	33.00 ±0.07
		F = 81.28		F = 59.96	F = 124.76	-	-
		P<0.01		P<0.01	P<0.01		

on single side possessed sufficient efficacy for management of *C. maculatus* (>75%). Treatment of the bioinsecticide saved 31% loss of weight in Bengal gram and 64% loss of weight in green gram. The storage quality of insect accessed as percentage of grains with holes and percentage of grains with eggs after storage duration of six months was within the acceptable values. The incorporation of storage bag can offer high degree of protection to storage grains and its lower cost could apprehensively reduce the burden of lost produce on the farmer due to improper storage facilities. Incorporating the storage bag with bioinsecticides hence proves to be cost effective, affordable and easy to adopt methodology of storage specially in developing nations where bag storage is still predominant.

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