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Integrated Management of *Callosobruchus maculatus* (Fab.) in Mung bean Stored as Seed

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Abstract: Bruchids causes quantitative and qualitative losses estimated to about 87 to 100 per cent within storage duration of 3-6 months. In order to manage the bruchid, *Callosobruchus maculatus* in stored mung bean seed studies were conducted at Punjab Agricultural University, Ludhiana. Five Kg mung bean seeds were treated with treatment combinations of neem oil (0.50-1.50%) and dharek (*Melia azedarach* L.) kernel powder (1.00-3.00%), later packed in deltamethrin 2.8 EC (0.025-0.075%) treated jute bags after release of 20 pairs of freshly emerged adults of *C. maculatus*. In order to optimize the test process conditions these bags were stored for 6 months under ambient laboratory conditions i.e. temperature (29.5 to 31.0°C) and relative humidity (43 to 75%) to observe seed damage (%), weight loss (%), colour change, moisture content (%), protein content (%) and germination (%) at an interval of 2, 4 and 6 months in disposable sets. The optimized condition was compared with zerofly bags, existing PAU recommendation along with untreated control. Numerical optimization showed that if mung bean seeds treated with neem oil (0.55%), dharek kernel powder (2.21%) and packed in deltamethrin 2.8 EC (0.055%) treated jute bags, may be stored safely for 2.50 months. The optimized conditions showed least seed damage (0.01%) and seed weight loss (0.01%) along with less colour change (2.14), moisture content (10.15 %) and maximum protein content (27.12%), seed germination (89.67%). Moreover, zerofly bags and metallic bin stored seeds also proved to be effective to store mung bean seeds.

Keywords: Mungbean, Botanicals, Deltamethrin, *Callosobruchus maculatus*, Quality parameters

Among various pulse crops, mung bean, *Vigna radiata* (L.) is an important crop that fits well in diverse cropping systems and is cultivated during summer and *Kharif* seasons across Northern India. The production of this crop has increased over the years but still relies on other countries for meeting its domestic need. One of the reason behind this is the post-harvest losses due to insect pest which alone may account to 10-50 per cent. Among various stored grain insects, Bruchids belonging to the order Coleoptera have been associated with the seeds of leguminous plants. The bruchid, *Callosobruchus maculatus* (Fabricius) is a cosmopolitan and polyphagous pest of economically important legume crops such as cowpea, lentil, mung bean and black gram. This insect pest has been can cause quantitative and qualitative losses estimated to about 87 to 100 per cent within storage duration of 3-6 months, leading to lesser returns to small scale farmers (Ojiako and Adesiyun 2013). Control of this insect pest can be achieved by synthetic chemicals. However due to hazards caused by these chemicals to plants, man and the environment there has been a move to search for alternative using plant materials such as bio pesticides to control *C. maculatus* (Yusuf et al 2011). Due to non-phytotoxic and easy bio-degradable, plant derivatives have a great potential in pest management. Thus, the present study was planned with an objective to develop an integrated management approach for pulse beetle in mung bean stored for seed purpose.

MATERIAL AND METHODS

The present investigations were conducted at Punjab Agricultural University (PAU), Ludhiana to study the integrated effect of different practices to manage *C. maculatus* in mung bean stored for seed purpose. The recommended mung bean var. TMB 37 were procured from the Farm of Director (Seeds), PAU, Ludhiana and dharek (*Melia azedarach* L.) kernels were obtained from Agroforestry Research Farm, PAU, Ludhiana which were tray dried, later grounded into fine powder by using electric mixer grinder. The neem oil and jute bags were procured from local market. The mung bean seeds used for conducting the experiments were disinfested at 60ºC temperature for an hour to make them free from any other insect infestation. The rearing of *C. maculatus* was also done on 100 gm disinfested mung bean seeds by releasing 10 pairs of adults in a jar and kept in an incubator at 29±2ºC and 70±5% relative humidity to get the pure culture for the experiments. After 48 hours, these adults were removed and the grains were kept as such for about 25 days for the emergence of adults and were used for conducting the experiments. The neem oil (0.50-1.50%)

and *M. azedarach* kernel powder (1.0-3.0%) were used to treat mung bean seeds along with deltamethrin 2.8 EC (0.025-0.075 %) was used to treat jute bags. The experiments were conducted using 3 factors Box-Behnken design of RSM for all the selected treatments (Design-Expert software version 8.0.3.1, Stat-Ease Inc., Minneapolis, U.S.A). The independent variables with three different levels viz. -1, 0 and +1 (Table 1) which formed a total of 27 different

Table 1. Independent variables and their levels

Independent variables	Symbol		Levels	
	Coded	Un-coded	Coded	Un-coded
Deltamethrin 2.8EC	X_1	A	-1 0 +1	0.025% 0.050% 0.075%
Neem Oil	X_2	B	-1 0 +1	0.50% 1.00% 1.50%
Dharek kernel Powder	X_3	C	-1 0 +1	0.50% 1.00% 1.50%
Storage Periods (months)	X_4	D	-1 0 +1	2.00% 4.00% 6.00%

Table 2. Quality of mung bean seeds for response surface analysis

Process variables				Product quality responses					
Deltamethrin 2.8 EC (%)	Neem oil (%)	Dharek kernel powder (%)	Storage methods (Months)	Seed damage (%)	Seed weight loss (%)	Colour Change	Moisture content (%)	Seed germination (%)	Protein content (%)
0.025	0.50	2.00	4	0.2	0.13	3.67	10.20	88.67	24.10
0.075	0.50	2.00	4	0.0	0.0	2.31	10.30	88.33	26.88
0.025	1.50	2.00	4	0.0	0.0	3.37	10.10	84.00	25.24
0.075	1.50	2.00	4	0.0	0.0	3.27	10.40	84.00	24.65
0.050	1.00	1.00	2	0.0	0.0	2.65	10.30	88.00	25.32
0.050	1.00	3.00	2	0.0	0.0	3.11	10.70	89.67	26.27
0.050	1.00	1.00	6	0.1	0.05	2.24	10.40	79.00	24.04
0.050	1.00	3.00	6	0.0	0.0	2.46	10.20	78.00	21.11
0.025	1.00	2.00	2	0.0	0.0	2.25	10.20	88.00	26.19
0.075	1.00	2.00	2	0.0	0.0	2.27	10.20	87.33	25.77
0.025	1.00	2.00	6	0.0	0.0	2.44	10.80	77.00	23.64
0.075	1.00	2.00	6	0.0	0.0	2.88	10.80	76.67	25.85
0.050	0.50	1.00	4	0.0	0.0	2.99	10.20	88.00	26.61
0.050	1.50	1.00	4	0.0	0.0	2.84	10.20	83.67	25.14
0.050	0.50	3.00	4	0.0	0.0	3.20	10.30	89.00	24.10
0.050	1.50	3.00	4	0.0	0.0	3.90	10.10	83.00	23.00
0.025	1.00	1.00	4	0.0	0.0	3.48	10.20	87.33	23.64
0.075	1.00	1.00	4	0.0	0.0	3.30	10.40	86.33	24.90
0.025	1.00	3.00	4	0.0	0.0	3.50	10.20	86.00	21.48
0.075	1.00	3.00	4	0.0	0.0	2.22	10.20	86.00	25.26
0.050	0.50	2.00	2	0.0	0.0	2.14	10.10	89.67	26.04
0.050	1.50	2.00	2	0.0	0.0	2.96	10.25	87.66	26.24
0.050	0.50	2.00	6	0.0	0.0	2.42	10.30	80.33	24.90
0.050	1.50	2.00	6	0.0	0.0	4.20	10.20	73.33	25.94
0.050	1.00	2.00	4	0.0	0.0	2.64	10.20	86.33	28.35
0.050	1.00	2.00	4	0.0	0.0	2.92	10.20	85.33	25.50
0.050	1.00	2.00	4	0.0	0.0	2.39	10.10	86.00	25.32

combinations (Giles et al 2004) having three replicates of the center point (Table 2). Later 5 kg mung bean seeds were exposed to selected treatment combinations for each bag and 20 pairs of freshly emerged adults of *C. maculatus* were released into it. These bags were kept for 6 months for storage studies under ambient conditions i.e. temperature (29.5 to 31.0^{o}C) and relative humidity (43 to 75%). Various observations such as grain damage (%) based on 1000 grains, grain weight loss (%) as per given by Adams and Schulten (1978), colour change measurement by using Colour Reader CR-10 Konica Minolta Sensing Inc. with the equation given by Gnanasekharan et al (1992), moisture content (%) in grains by hot air oven method (AOAC 1984), protein content (%) by using AOAC (2000) and germination as per the equation given by Patel (2001) were recorded for mung bean seeds at regular interval of 2, 4 and 6 months in disposable sets. The design was taken from response surface designs and it fulfills most of the requirements needed for optimization of the best method for the management of insects with targeted goals of less seed damage, less weight loss, less change in colour, less moisture content, more protein content and more germination of mung bean seeds. The best treatment (optimized condition) was compared with zerofly bags, existing PAU recommendation (7cm layer of sand on top of mung bean seeds stored in metallic bins) along with untreated control.

RESULTS AND DISCUSSION

The experimental data related to management of *C. maculatus* in mung bean stored for seeds were presented in Table 2. F-value and the final equation of the fitted model for the selected parameters is depicted in Table 3 and 4 along with the R^2 values. The regression models for colour change, moisture content (%), protein content (%) and germination (%) were significantly high witnessing high correlation coefficient (R^2 = 0.81, 0.79, 0.80 and 0.98, respectively). None of the models showed significant lack of fit (P > 0.01), indicating that all the second-order polynomial models correlated well with the measured data. All the parameters showed high adequate precision. The studies indicated closer the value of R^2 to the unity, the better the empirical model fits the actual data.

Seed damage and seed weight loss (%): The seed damage ranged from 0.0 to 0.2% and seed weight loss ranged from 0.0 to 0.13% in various experimental combinations of deltamethrin, neem oil, dharek kernel powder along with the storage period (Table 2). There was no seed damage at 2, 4 and 6 months of storage in various combinations except where jute bags were treated with 0.025

Table 3. Statistically analyzed data for the selected responses

Source	F-value			
	Colour change	Moisture content (%)	Seed germination (%)	Protein content (%)
Fitted model	Quadratic	Quadratic	Quadratic	Quadratic
A-Deltamethrin (%)	1.23 (0.289)	0.84 (0.378)	0.92 (0.355)	7.58 (0.017)**
B- Neem oil (%)	2.40 (0.147)	0.00 (1.000)	135.52 (<0.0001)**	0.55 (0.474)
C- Dharek kernel powder (%)	2.51 (0.139)	0.052 (0.823)	0.073 (0.7909)	6.62 (0.024)**
D- Storage period (months)	20.64 (0.0007)*	27.72 (0.0002)*	734.99 (<0.0001)**	9.98 (0.008)*
AB	3.85 (0.073)	0.00 (1.000)	0.059 (0.813)	3.18 (1.001)
AC	10.18 (0.008)*	0.63 (0.443)	0.51 (0.490)	1.78 (0.207)
AD	1.10 (0.315)	0.00 (1.000)	0.059 (0.813)	1.93 (0.189)
BC	0.86 (0.373)	0.63 (0.443)	1.41 (0.257)	0.04 (0.848)
BD	1.06 (0.323)	0.63 (0.443)	12.60 (0.004)**	0.20 (0.665)
CD	0.05 (0.832)	1.41 (0.257)	3.61 (0.082)	4.21 (0.063)
A^2	3.21 (0.098)	3.64 (0.080)	7.92x10-^{3} (0.931)	0.69 (0.421)
B2	3.46 (0.088)	5.82x10^{-3} (0.940)	7.92x10-^{3} (0.931)	0.59 (0.458)
C2	0.43 (0.525)	0.37 (0.553)	2.87 (0.116)	8.34 (0.014)*
D2	0.069 (0.797)	9.32 (0.010)*	107.29 (<0.0001)**	0.090 (0.769)
Std. Dev.	0.32	0.13	0.988	0.95
C.V. %	11.24	1.22	0.937	3.80

The data in parenthesis are the p-values
*significant at 1 %level of significance (p<0.01),** significant at 5 %level of significance (p<0.05)

and 0.050% deltamethrin and mung bean seeds were treated with neem oil (0.50 & 1.00%) and dharek kernel powder (2.00 & 1.00 %) at 4 and 6 months of storage, respectively (Table 2). Sharma et al (2019) also observed that neem oil @ 10ml/kg seed completely inhibited the oviposition, adult emergence and seed damage, which may be due to growth disruption and repellant property of azadirachtin present in neem (Schmutterer 1990). Zafar et al 2018 concluded that neem powder to be effective which also corroborates with the present findings where additional doses of dharek powder did not show any effect on insect mortality, while lower dose of neem oil proved to be effective (Table 2).

Colour change: The effect of the independent variables on colour change ranged from 2.14 to 4.20 irrespective of the experimental combinations (Table 2). The effect of storage period was significantly positive witnessing that the increase in storage period leads to more change in colour of mung bean seeds. There was non-significant effect of deltamethrin 2.8 EC, neem oil and dharek kernal powder on colour change. Ogendo et al (2004) also observed that the grain colour and odour were unaffected by the botanicals. The fitted model was quadratic and linear term of storage period as well as product term of deltamethrin and dharek kernel powder was significant and negative on colour change of mung bean seeds. The storage period (month) witnessed the significantly higher effect in comparison to other treatments with F-value (20.64) (Table 3). Therefore, there exists positive relationship between storage period on colour change. The regression equation for the colour change of mung bean seed is shown in Table 4.

Moisture content (%): The moisture content ranged from 10.10 to 10.80% irrespective of the experimental combinations (Table 2). Maximum moisture content was observed at six months of seed storage, when jute bags were treated with 0.025 & 0.075% deltamethrin 2.8 EC and mung bean seeds treated with 1.00% neem oil and 2.00% dharek kernel powder, while, minimum moisture content in the mung bean seeds were observed at 2 months of storage in various combinations of deltamethrin, neem oil and dharek kernel powder. The effect of storage period was significantly positive witnessing that the increase in storage time leads to more change in moisture content of mung bean seeds. This fluctuation in moisture content might be due to the variation in an atmospheric humidity, as the seeds are hygroscopic in

Table 4. Adequacy of model fitted

Parameters	Fitted models	Model equation	R^2	P value
Colour change	Quadratic	$=2.66-0.10*A+0.14*B-0.15*C+0.42*D+0.32*AB-0.51*AC+0.17*AD+0.15*BC-0.17*BD-0.035*CD+0.25*A^2+0.26*B^2-0.091*C^2+0.037*D^2$	0.81	0.01
Moisture content (%)	Quadratic	$=10.20+0.033*A+0.000*B+8.333\times10^{-3}*C+0.19*D+0.000*AB-0.050*AC+0.000*AD-0.050*BC-0.050*BD-0.075*CD+0.10*A^2+4.167\times10^{-3}*B^2-0.033*C^2+0.17*D^2$	0.79	0.02
Protein content (%)	Quadratic	$=25.39+0.75*A-0.20*B-0.70*C-0.86*D-0.84*AB+0.63*AC+0.66*AD+0.092*BC+0.21*BD-0.97*CD-0.34*A^2+0.31*B^2-1.18*C^2+0.12*D^2$	0.80	0.01
Germination (%)	Quadratic	$=85.89-0.19*A-2.36*B-0.055*C-5.50*D+0.085*AB+0.25*AC+0.085*AD-0.42*BC-1.25*BD-0.67*CD-0.027*A^2-0.027*B^2+0.52*C^2-3.15*D^2$	0.98	<0.0001

Table 5. Optimum values of process parameters and responses for mung bean stored as seeds

Process parameters	Target	Experimental range		Optimum values
		Minimum	Maximum	
Deltamethrin (%)	Range	0.025	0.075	0.055
Neem oil (%)	Range	0.50	1.50	0.55
Dharek kernel powder (%)	Range	1.00	3.00	2.21
Storage period (Months)	Range	2	6	2.39
Responses				
Colour change	Minimize	2.14	4.20	2.14
Moisture content (%)	Minimize	10.10	10.80	10.15
Germination (%)	Maximize	73.33	89.67	89.67
Protein content (%)	Range	21.11	28.35	27.12

The desirability level was 0.975

nature and they absorb moisture from atmosphere. Similar results were obtained by Beedi et al (2018) and Angelovic et al (2018). There was non-significant effect of deltamethrin 2.8 EC, neem oil and dharek kernel powder on moisture content. Based on F-value, the storage period witnessed the significantly higher effect in comparison to other treatments (Table 3). The regression equation for the moisture content is shown in Table 4.

Germination (%): Seed germination varied from 73.33 to 89.67 % irrespective of the experimental combinations (Table 2). The maximum seed germination was at 2 months of storage where jute bags were treated with deltamethrin @ 0.050% and seeds were treated with 1.00 & 0.50% neem oil and 3.00 & 2.00% dharek kernel powder, followed by different experimental combinations used for different months of storage. The effect of neem oil and storage period was significantly negative witnessing that the increase in neem oil percentage and storage period reduced the germination percentage of mung bean seeds. There was non-significant effect of deltamethrin 2.8 EC and dharek kernel powder on germination percentage. The fitted model was quadratic and linear term neem oil, storage period, product term neem oil and storage period and quadratic term storage period was negative on seed germination percentage. The neem oil percentage and storage period witnessed the significantly higher effect in comparison to other treatments (Table 3). Rath et al (2013) observed that the germination of green gram seeds was inhibited with neem oil treatment and the same was observed in case of shoot and root length, fresh and dry weights. The study indicated that the plant protectors like neem oil is to be administered in very low concentrations and for shorter duration since higher concentration and duration is affecting all the germination parameters significantly. Kadam et al (2013) also observed that neem oil @ 5ml/kg seed inhibit the seed germination up to 80.33% at 9 months of storage period. However, a decline in germination percentage was observed in all the treatments with advancement in the storage period, which may be attributed to the phenomenon of ageing and due to depletion of food reserves, decline in synthetic activity (Beedi et al 2018)

Protein content (%): The protein content ranged from 21.11 to 28.35 % (Table 2). The maximum protein content was at 4 months of storage when jute bags were treated with deltamethrin 2.8 EC @ 0.050% and seeds were treated with neem oil @ 1.00% and dharek kernel powder @ 2.00 %, while minimum at 6 months of storage when jute bags were treated with deltamethrin 0.050% and seeds were treated with neem oil @ 1.00% and dharek kernel powder @ 3.00 %. The effect of storage period was significantly negative witnessing that the increase in storage period leads to decrease in protein content of mung bean seeds (Table 3). There was positive significant effect of deltamethrin 2.8 EC treated jute bags on protein content, which may be due to that the seeds stored in the treated bags remain unaffected with the insect attack and there was negative significant effect of dharek kernel powder on protein content of mung bean seeds, which showed that dharek kernel powder may affect the seed properties. There was non-significant effect of neem oil percentage on protein content, which corroborates with the study conducted by Sharma et al (2022).

Optimum treatment conditions for the storage of mung bean seed and its comparison with zerofly bag as well as metallic bin stored mung bean seeds: The various independent parameters for storage of mung bean seeds were optimized using numerical optimization technique (Table 5). There was negligible seed damage and weight loss were found in almost all the experimental combination, so they were not taken for optimization. Thus, the main criteria for various responses to be undertaken for optimization include minimum colour change, minimum moisture content, maximum germination and protein content to be in the range. In order to optimize deltamethrin for treating jute bags, while neem oil and dharek powder for treating mung bean seed to enhance their storage life, an equal importance was given to all the parameters and responses. The zone of optimization for various parameters

Table 6. Comparison between different storage practices used to store mungbean seeds

Process parameters	Responses					
	Seed damage (%)	Seed weight loss (%)	Colour change	Moisture content (%)	Germination (%)	Protein content (%)
Optimum values of D:NO:DKP:SP (0.055:0.55:2.21:2.50)	0.01	0.01	2.14	10.15	89.67	27.12 (0.11)
Zerofly bag (2 months)	0.40	0.26	1.38	10.30	91.00	26.35 (2.94)
Metallic drum stored seed (2 months)	0.00	0.00	2.17	10.40	95.00	27.11 (0.15)
Untreated control (2 months)	2.00	0.41	1.95	10.90	78.50	25.98 (4.30)

D= Deltamethrin, NO= Neem Oil, DKP= Dharek Kernel Powder, SP= Storage period
* values in parenthesis are the percent change in protein over period of time
Fresh sample: Protein= 27.15% , Moisture content = 10.20%, Germination: 97%

depicts that the 0.055% deltamethrin 2.8 EC for treating jute bags and 0.55% neem oil as well as 2.21% dharek kernel powder for seed treatment, under these conditions mung bean seed may store for 2.50 months witnessing optimized predicted values i.e. colour change (2.14), moisture content (10.15%), germination (89.67%) and protein content (27.12%) with desirability of 0.975. Thus, the optimized values show that mung bean seeds may be stored for 2.50 months without much change in tested responses. The optimum values of seed damage (0.01 %) and seed weight loss (0.01 %) in treated combinations was compared with zerofly bag, metallic bin, untreated control stored mung bean seeds for 2 months indicated that there was 0.40, 0.00, 2.00 % seed damage and 0.26, 0.00, 0.41% seed weight loss, respectively, which corroborates with study conducted by Ahmad et al (2015), where use neem leaf powder showed strong detrimental effect on pulse beetle in mung bean. Zerofly bags showed good response against test insect, similar results were observed by Mutungi et al (2014), where triple layered hermetic bags halted multiplication of *C. maculatus.* Similarly, the results of metallic bin stored pulses by using top sand layer was similar to Swamy et al (2018). The moisture content in seeds stored with optimized parameters was 10.15%, while 10.30, 10.40 and 10.90 % observed in zerofly bag, metallic bin with sand layer and untreated control stored seeds, respectively (Table 6) which is the safe limit as per observed by Kadam et al (2013). Mutungi et al (2014), also observed that the moisture content of grain stored in triple layered hermetic bags and PP bags remained below 12%. The moisture content in control bags also remained stable which may be due to atmospheric conditions. Seed germination was 89.67, 91.00, 95.00 and 78.50% in treated seeds with optimized parameters, zerofly bags, metallic bin with sand layer and seed stored under untreated control, respectively at 2 months of storage. The same has been reported by Rath et al (2013), where germination of green gram seeds were inhibited with neem oil and by Mutungi et al (2014) that germination of mung bean stored in PICS (triple layered hermetic) bags dropped marginally to 84.19% whereas in pigeon peas dropped 78.42% after six months of storage and was not affected when seed stored by using sand layer (Swamy et al 2018). The change in protein content was very less in treated seeds with optimized parameters followed by metallic drum, zerofly bag and untreated control stored mung bean seeds at 2 months of storage (Table 6). There was very less reduction of protein content was observed in untreated control, which may be due to the eggs, egg cases, excretory products left behind on removal of larval, pupal and adult stages of *C. maculatus* before analysis and corroborates with the study conducted by Sharma et al 2022.

CONCLUSION

The response surface methodology was appropriate for optimization of process conditions for storage of mung bean seeds. The optimized conditions for storage of mung bean seed were found to be deltamethrin 2.8EC @ 0.055% for treating the jute bags along with mung bean seed treated with neem oil @ 0.55% as well as dharek kernel powder @ 2.21% and thus, seed may be stored for 2.39 months. Under these conditions, optimal values of responses were for colour change-2.14, moisture content-10.15%, germination-89.67% and protein content-27.12%. The use of insecticides for treating the seeds during storage created various issues like development of pest resistance, resurgence and residue effects on grains besides ill effects on human and animal. Thus, the ecofriendly pest management strategy is the only solution to avoid above said situations. In view of that neem oil and dharek kernel powder used to treat mung bean seeds and deltamethrin used to treat empty gunny bags against *C. maculatus* under laboratory conditions. The use of zerofly bags and metallic drum stored seeds also proved effective to store mung bean seeds. The finding of present work revealed that mung bean seeds may be stored for 2.50 months without much change in tested responses.

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