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Efficacy of Natural Plants Extract and Ethnomedicinal Dilution on Bruchid (*Callosobruchus maculatus*) Management in Storage Mungbean [*Vigna radiata* (L.) Wilczek]

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ABSTRACT

Background: Storage of pulse grain is a severe on-going problem for farmers, traders and also the consumers. Mungbean [*Vigna radiata* (L.) Wilczek] is one of such vital protein rich pulse crops of Indian origin referred to as poor man's meat. Bruchids (*Callosobruchus maculatus*) (Coleoptera: Bruchidae) are the major polyphagous storage pest of mungbean which causes the substantial post-harvest qualitative and quantitative loss, especially in long time storage condition. This loss directly affects the agricultural economy and also the human health.

Methods: To find a solution, we first screened a large variety of mungbean genotypes against bruchid attack and narrowed down to six most potential varieties with highest resistance to bruchids. The present study is intended to manage pest control in post-harvest storage condition using easily available natural and eco-friendly treatment materials against the much-used chemical pest control materials. We have instinctively chosen eight treatment materials that are not used before except one in any earlier but similar studies of pest control management on other grains. Apart from direct natural sources of these treatment materials, we have used two ethnomedicinal dilution and evaluated their management efficacy as well.

Result: The study highlights a new promising eco-friendly approach for bruchid management either through the 'Homeo-Bruchid Interaction' or through the 'Easily Available Homemade Remedies'. Our results are based on the crude extracts. In few specific situations, we have tried to give possible molecular level interactions that provide the bruchid resistance. However, further investigation is deemed necessary to properly assess the quality components of treating seed as well as identifying particular properties that are responsible for bruchid resistance and to confirm the level of human safety based on their bioactivity before commercialization.

Key words: Bruchid, Ethnomedicinal dilution, Management, Mungbean, Natural treatment, Pest resistance.

INTRODUCTION

The progress of the world economy is partially dependent on the agricultural product, particularly to the extent of importing and exporting of higher quality seeds. The entire flow requires several levels of storage facilities. However, the storage of pulse grain is a severe problem for farmers, traders and also the consumers. Bruchids are well known to settle in each level of the pulse environment, from introductory field invasion through all level of storage and circulation (Srinives *et al*., 2007). After the massive damage during storage, pulse grains lose their weight, conservation of nutritional quality, seed viability and also create the environment for secondary infestation by the fungus (Srinives *et al*., 2009). Mungbean [*Vigna radiata* (L.) Wilczek] is very adaptable pulse crop which can grow in low rainfall and high temperature. It is the cheapest source of vegetable protein. The conventional farmers are trying to keep a small percentage of seeds for their family to use as staple food and rest of the maximum harvested seed materials are stored into their godown where the cross infestation is found in most of the cases (Stathers *et al*., 2002). Bruchids damage over 85% of the mungbean seeds during storage (Kosini *et al*., 2015). So, this is a major constraint to increase the production and consequently to contribute to colossal food shortage in tropical and subtropical countries of the world (Rees, 2004). The low yielding cultivars are less susceptible to bruchid attack compare to high yield cultivars and for that reason, the traditional farmers are obligated to cultivate low yield genotype (Shazia *et al*., 2006). For increasing the mungbean productivity and consumption, not only is it necessary to cultivate the high yielding genotype but also it is required to prevent the bruchid infestation. The current knowledge and means of management of bruchid control is limited. Application of synthetic pesticides in any forms

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against the bruchid attack is very effective but expensive for farmers. Not only this, it poses risk to safe use due to lack of technical knowledge and sometimes it might be carcinogenic (Ntoukam *et al*., 2000). In addition, in recent years, storage pests have evolved the pesticide tolerant strains due to increased use of non-degradable pesticides which disturbed the ecological flora and fauna. The ultimate target of any storage management strategy should be safe and longtime stockpiling of grains without compromise of their quality. So, an alternative outlook for the bruchid attack control is the usage of natural products such as botanical extracts or powder, cow dung with different level of effectiveness (Rotimi and Ekperusi, 2012). The use of plant materials is a traditional local method in Asia and Africa to protect the stored grains from pest attack. Various plants are listed in GRAS (Generally Recognized as Safe) which is approved by the FDA (Food and Drug Administration) and EPA (Environment Protection Agency) in the United States of America (Prakash *et al*., 2012). In this modern civilization, medicine has been a mandatory intake after food to survive in daily life. The worldwide people are now self-conscious about their health and try to use organic food. Samuel Hahnemann had established in 1796 (WHO, 2007) a parallel medical field, called Homeopathy and proposed the philosophical basis of the field as “similia similibus curentur (like cures like)”. According to Hahnemann, a small dose of homeopathic dilution activates the vital force which increased the body autoimmune system against the disease. Nowadays, homeopathy is widely spread in all areas of WHO due to its low cost and better remedies and it is also been incorporated into the plant and animal protection.

In this study, we aim to assess two alternative treatments for better managing the bruchid infestation. Firstly, we have evaluated the use of extracts of easily available plant materials. Secondly, we have used homeopathic dilution and evaluated their management efficacy to protect the mungbean seeds from the bruchid infestation.

MATERIALS AND METHODS

Experimental materials

Six mungbean genotypes *viz*. SML-302, Pusa Baisakhi, Panna, Howrah local, PDML-13-11 and HUM-16 were collected from National Bureau of Plant Genetic Resources (NBPGR – New Delhi), Indian Institute of Pulses Research (IIPR -Kanpur), Pulses and Oil seeds Research Station (Baharampore, West Bengal) and local District Collection (West Bengal). The first part of this work, we screened fifty-two mungbean genotypes and from that work six genotypes were selected based on their moderate to high susceptibility index (Ghosh *et al*., 2022). Howrah local was selected due to see the treatment effect on susceptible genotype.

Preparation for the herbal treatment solution

The list of plant materials for herbal treatment and homeopathy medicine is shown in Table 1. The bay leaves, fenugreek seeds, cinnamon bark and black cumin seeds were bought from the local market. The holy basil inflorescence and citrus fruit peel were collected from home. All the collected treatment materials were dried at 35±2°C for 7 days and then each material was grounded to the fine powder. Then we prepared 10% ethanolic solution from 99.9% ethanol. The 5 g powder of each material was put into 100 ml of 10% ethanol to prepare 5% (w/w) treatment solution and stored in the amber bottle. These treatment solutions were kept into the shaker overnight for maximum extraction of the herbal components. Next day, the extracted solutions were filtered using Whatman No.1 filter paper and kept in normal condition to be used as a stock treatment solution. The protocol for the preparation of the stock solution was according to Radha and Sushila, (2014) with significant modifications.

Homeopathy medicine Arsenicum album 30 CH (centesimal Hahnemannian dilutions) and Thuja occidentalis 30 CH of Dr. Reckeweg company bought from the homeopathy medicine shop. The 0.01% treatment solution (alcohol based) was prepared according to modification method of Hanif and Dawar (2016) and kept into the normal condition. The 10 ul of Thuja 30 CH and Arsenicum 30 CH were dissolved into 100 ml of 10% ethanol.

Seed treatment

Fifty seeds were collected from the six genotypes for each treatment and the respective control and dried them at 45°C for 24 hours in order to destroy bruchid’s egg or larvae if any present in the seeds. Then, 0.5 ml of each stock treatment solution was added into the six different test-tube for different treatment and control. Here, we used two types of control. The first control was prepared without adding any solution as untreated seed and in second control 0.5 ml of 10% ethanol was added into six separate test tube for use

Table 1: List of treatment plants and parts used against bruchid attack.

Sl. no.	Common name	Scientific name	Family	Parts used
T1	Bay leaf	*Cinnamomum tamala*	Lauraceae	Leaf
T2	Holy basil	*Ocimum sanctum*	Lamiaceae	Inflorescence
T3	Fenugreek (Methi)	*Trigonella foenum-graecum*	Fabaceae	Seed
T4	Sweet orange	*Citrus sinensis* osbeck	Rutaceae	Fruit peel
T5	Cinnamon	*Cinnamomum verum*	Lauraceae	Bark
T6	Black cumin	*Nigella sativa*	Ranunculaceae	Seed
T7	Thuja 30 CH	*Thuja occidentalis*	Homeopathy medicine	
T8	Arsenic 30 CH	*Arsenicum album*	Homeopathy medicine	

as a control for each genotype and kept into room temperature for two hours. After that, the treated seeds and control seeds of each genotype were again dried overnight.

Experimental design

Here, we have particularly used Bruchid, *Callosobruchus maculatus*. According to Raina (1970) and Beck and Blumer (2014), the male and female bruchid were identified. *C. chinensis* and *C. maculatus* have some common features. In *C. maculatus* both antennae are serrate. But the most distinctive feature is white spot showed on the elytra. The male bruchid is slim and long where female bruchid large and short size. Twenty pairs of adult male and female bruchid (1:1) were collected from the State seed testing laboratory, West Bengal and transferred them to set up culture jar in the B.O.D incubator for maintained the temperature and humidity (28±2°C, 65±5% respectively). A glass jar (13 cm height × 23 cm diameter) was used as set up cultured jar where put into 100 gm of mungbean seeds for culturing the bruchid. After four weeks, newly cultured bruchids were used for this experiment. The seeds of six mungbean genotypes were evaluated in two consecutive the year of 2017 and 2018. The present experiment was conducted in the B.O.D condition through the completely randomized design (CRD) with three replications. The treated mungbean seeds of each genotype were weighed before forced infestation and the method of 'No Choice Test' followed as per Somta *et al.* (2008). The seeds of six genotypes were put into the plastic container (4 cm height × 12 cm diameter) along with two pairs of male and female bruchids (1:1) for oviposition and infestation purposes.

Data collection

The data were recorded from three replications on different parameters against bruchid attack *viz*. initial seed weight (ISW), number of eggs laid (NOEL), number of adults emerge (NOAE), percentage of egg hatched (POSD), number of seed damage (NOSD), percentage of seed damage (POSD), percentage of seed weight loss (POSWL), mean development days (MDD), percentage of bruchid resistance (POBR) and bruchid susceptibility index (BSI). In our previous article (Ghosh *et al*., 2020), we elaborately described about the calculation method of these parameters. The bruchid susceptibility index categorized into group according to Dobie (1974) with minute modification. *viz*:

BSI 0 to 3-Resistant,
3.1 to 6-Moderately Resistant,
6.1 to 8-Moderately susceptible,
8.1 to 10-Susceptible,
and > 10-Highly susceptible.

Statistical analysis

The collected data of different parameters of bruchid attack for management in selected mungbean genotypes were analyzed through the software of SPAR 2.0 and IBM SPSS 20.0. Analysis of Variance (ANOVA) was done through the software SPAR 2.0 whereas the mean performance analysis was performed through the Duncan's multiple range test (DMRT), significance at P>0.05.

RESULTS AND DISCUSSION

The analysis of variance (ANOVA) is shown in Table 2. This shows significant differences of the selected parameters among all traits. The interaction (Genotype × Treatment) shows that higher significant difference is found in percentage of egg hatched (156.65), percentage of seed weight loss (148.21), percentage of seed damage (124.86), percentage of bruchid resistance (124.86), number of adult emergence (81.06) and number of eggs laid (74.83). The low significant difference is found in bruchid susceptible index (0.81) compared to number of seed damage (31.22) and mean development days (11.94).

The evaluation of the management of bruchid control through herbal treatment is shown in Table 3. The treatments are identified as follows: Bay leaf (T1), holy basil (T2), fenugreek (T3), sweet orange (T4), cinnamon (T5), black cumin (T6), thuja 30 CH (T7) and arsenic 30 CH (T8). Among the six selected genotypes, four genotypes were exhibited resistance through the application of different treatments. The genotype SML-302 treated through T2, T4, T6 and T7 shows less NOEL (24.59, 27.60, 28.89 and 24.92 respectively), NOAE (10.30, 11.04, 11.57 and 11.28 respectively), NOSD (8.18, 9.33, 9.76 and 8.44 respectively), POSWL (11.36, 8.61, 7.14 and 6.59 respectively) with longer MDD (36.39, 34.99, 36.34 and 35.01 respectively), high POBR (83.64, 81.35, 80.48 and 82.32 respectively), low BSI (2.78, 2.98, 2.93 and 3.00 respectively). SML-302 is exhibited as bruchid resistant after the treatment through T2, T4, T6 and T7. Treatment applications T2, T3, T6 and T7 for mungbean genotype Pusa Baisakhi display less NOEL (30.76, 30.21, 25.13 and 26.26 respectively), NOAE (11.93, 13.41, 11.75 and 9.57 respectively), NOSD (10.49, 10.40, 9.09 and 7.55 respectively), POSWL (18.78, 16.30, 11.89 and 10.58 respectively) with long MDD (36.49, 38.40, 37.80 and 33.01 respectively), low bruchid susceptibility index (2.95, 2.93, 2.82 and 2.97 respectively) and is observed as bruchid resistant compared to the untreated as well as control. In genotype Panna, among all the treatments, T1, T2, T3, T6 and T7 show less NOEL (30.38, 27.42, 31.18, 28.52 and 27.20 respectively), NOAE (11.91, 10.43, 12.37, 11.23 and 10.66 respectively), NOSD (10.30, 9.45, 9.79, 9.08 and 9.36 respectively), POSWL (19.89, 18.08, 14.89, 18.27 and 15.27 respectively), high MDD (36.51, 37.45, 34.66, 36.88 and 36.79 days respectively), high POBR (79.29, 80.31, 81.23, 81.22 and 81.51 respectively) with low BSI (2.95, 2.75, 2.92, 2.85 and 2.79 respectively) and is also identified as bruchid resistant compared to untreated and control. In genotype PDML-13-11, among all the treatments only T6 recorded less NOEL (21.83), NOAE (13.05), NOSD (10.70), POSWL (13.99), long MDD (37.62) with low BSI (2.97) and is found to be bruchid resistant compared to control, untreated and other treatment. The moderately resistant genotype HUM-16 exhibits resistance

Table 2: Analysis of Variance (ANOVA) showed the genotype × treatment interaction of different parameters against bruchid attack.

Source	df	NOEL	NOAE	POEH	NOSD	POSD	POSWL	MDD	POBR	SI
Genotype	5	1771.28**	1665.27**	2249.29**	806.63**	3226.54**	2339.43**	268.24**	3226.54**	18.16*
Treatment	7	879.479**	595.94**	186.42**	158.30**	633.20**	597.48**	201.84**	633.20**	12.26*
Genotype × Treatment	35	74.83**	81.06**	156.65**	31.22**	124.86**	148.21**	11.94*	124.86**	0.81*
Error	96	22.04	0.89	1.63	0.89	3.57	32.61	0.28	3.57	0.01
Total	143									

Note. **= Significant at P> 0.01 level, *= Significant at P>0.05

Note: NOEL: Number of egg laid, NOAE: Number of adult emerge, POEH: Percentage of egg hatched, NOSD: Number of seed damage, POSD: Percentage of seed damage, ISW-Initial seed weight, POSWL: Percentage of seed weight loss, MDD: Mean development days, POBR: Percentage of bruchid resistance, SI: Susceptible index.

only for T7 treatment compared to the untreated and control. The most susceptible genotype Howrah local did not show any resistance at all in any of the treatments. However, in all the genotypes, POBR is significantly increased through all the treatment compared to their control. All of these treatments show effective performance on the susceptible genotype Howrah local to reduce NOAE, NOSD, POSWL as well as reduce BSI and expressed as MR mungbean. Therefore, it is evident that among all the treatments, T2, T6 and T7 show better performance on preventing the bruchid attack in selected mungbean genotypes shown in Table 4 and Fig 1.

Evaluation of different herbal as well as homeopathic dilution treatment is shown their effect on preventing the bruchid attack in six selected mungbean genotypes. The treatment materials, particular the bay leaves (T1), fenugreek (T4), black cumin seeds (T6) and cinnamon bark (T5) are used extensively in most of the everyday cuisine in Indian subcontinent. In this experiment, treatments are prepared as 5% concentrated solutions. Tabu *et al*. (2012) reported that 2% and 5% seed dust treatment of *Azadirachta indica* and *Chenopodium ambrosiodes* suppressed the bruchid oviposition in chickpea cultivars. The Indian bay leaves (*Cinnamomum tamala*) is known as 'Tejpata' in Ayurveda and 'tamâlapattram' (dark tree leaves) in Sanskrit. It has aromatic constituents due to presence of essential oil such as linalool, beta-caryophyllene, eugenol *etc*. Among all genotypes, bay leaves show the better effect on Panna for reducing number of seed damage. Genotype Panna is exhibited as resistant through the treatment of bay leaves. The essential oil in bay leaf contains aromatic molecules. Therefore, it could be anticipated that the aromatic molecules might act as blocking agents and cause respiratory impairment for bruchid larvae. Poornasundari and Thilagavathy (2015) recorded that mungbean seeds treated with leaf powder of *Mentha arvensis* showed 100% mortality of bruchid particularly in species of *Callosobruchus chinensis*. The holy basil plants are found almost in every Indian house. The leaves of holy basil are also known as 'tulasi' and it very often used as traditional medicine in Ayurveda from the Vedic era as well as worship of 'Lord Krishna' in Hinduism. However, the inflorescences are not commonly used for medicinal purposes. In recent years, waste product management has become a trend in many fields such as from agriculture to engineering. Here, the extraction of holy basil inflorescence (T2) significantly reduced the bruchid oviposition, seed damage percentage, seed weight loss and increased the percentage of resistance in all the genotypes. Based on their low BSI value, genotype SML-302, Pusa Baisakhi, Panna is exhibited as bruchid resistant through the treatment of holy basil inflorescence (T2). The treatment of basil inflorescence reduced the number of adult emergence as well as increased the mean development days exhibited that high resistance ability in mungbean seeds which suggested that it might be due to the presence of secondary metabolites particularly the high

Table 3: Effect of different plant extracts and homeopathic dilution against different parameters of bruchid attack in different mungbean genotypes.

	NOEL	NOAE	POEH	NOSD	POSD	POSWL	MDD	POBR	BSI	Status
Genotype SML-302										
Untreated	30.81±2.24^{d-e}	14.05±2.23^{d}	45.90±1.83^{a-c}	12.33±2.40^{g}	24.66±4.80^{f}	19.83±2.38^{f}	29.38±2.54^{a}	75.34±4.80^{a}	3.90±1.19^{c}	MR
Control	29.92±2.03^{d}	13.18±2.15^{d}	44.05±2.33^{a-c}	12.06±1.18^{g}	24.11±2.36^{f}	20.70±5.13^{f}	30.39±1.13^{a}	75.89±3.36^{a-b}	3.69±1.01^{b-c}	MR
T1	30.26±1.15^{e}	13.14±1.16^{d}	43.43±1.15^{a-c}	11.63±2.07^{f-g}	23.27±1.35^{e-f}	17.58±6.81^{d-e}	35.85±3.21^{e}	76.73±1.35^{b-c}	3.12±0.72^{b}	MR
T2	24.59±3.27^{a}	10.30±3.14^{a}	41.89±3.26^{a-b}	8.18±1.36^{a}	16.36±2.12^{a}	11.36±4.80^{b-c}	36.39±2.16^{e-f}	83.64±3.12^{g}	2.78±0.31^{a}	R
T3	30.48±1.19^{e}	12.15±2.11^{b-c}	39.87±2.13^{a}	10.50±2.28^{c-d}	21.00±2.55^{c-d}	14.10±4.56^{c-d}	34.86±4.20^{c-d}	79.00±4.55^{d-e}	3.11±1.02^{b}	MR
T4	27.60±1.07^{b}	11.04±2.16^{a-b}	40.00±1.31^{a}	9.33±1.13^{b}	18.65±1.25^{b}	8.61±2.80^{a-b}	34.99±1.16^{c-d}	81.35±2.25^{e-f}	2.98±0.55^{a-b}	R
T5	29.36±3.11^{c-d}	12.62±3.26^{c-d}	42.97±2.68^{a-b}	11.27±2.52^{d-f}	22.55±1.46^{e-f}	18.68±6.30^{f}	32.37±2.03^{b}	77.45±3.63^{c-d}	3.40±1.42^{b}	MR
T6	28.89±2.04^{c}	11.57±1.06^{b-c}	40.04±1.16^{a}	9.76±1.26^{b-c}	19.52±1.51^{b-c}	7.14±1.08^{a}	36.34±3.28^{e-f}	80.48±3.51^{e}	2.93±0.86^{a-b}	R
T7	24.92±2.45^{a}	11.28±2.74^{a-b}	45.21±2.56^{b-c}	8.84±1.06^{a-b}	17.68±1.75^{a-b}	6.59±4.63^{a}	35.01±2.08^{e}	82.32±2.26^{f}	3.00±0.99^{a-b}	R
T8	27.61±1.38^{c}	13.17±1.65^{d}	47.78±2.91^{c}	10.77±1.85^{d-f}	21.54±1.70^{d-e}	13.74±2.55^{c-d}	33.73±4.05^{c}	78.46±1.70^{d}	3.32±0.58^{b}	MR
Mean	28.18±1.99	12.05±2.17	42.80±2.13	10.26±1.71	20.52±2.08	13.16±4.10	34.43±2.58	79.48±3.05	3.14±0.87	
	NOEL	NOAE	POEH	NOSD	POSD	POSWL	MDD	POBR	BSI	Status
Genotype Pusa Baisakhi										
Untreated	31.65±1.78^{d}	18.18±2.78^{f}	57.03±3.83^{f}	12.86±1.06^{d}	25.72±2.12^{e}	26.46±7.19^{h}	31.75±1.49^{a}	74.28±2.12^{a}	3.99±0.33^{c}	MR
Control	30.46±3.30^{c}	15.59±1.52^{e}	51.20±2.42^{f}	11.20±2.04^{c}	22.39±1.09	22.52±2.08^{f-g}	32.49±1.11^{a-b}	77.61±2.09^{b}	3.67±0.61^{c}	MR
T1	30.40±2.63^{c}	14.61±2.29^{d}	48.07±2.74^{e}	10.88±3.26^{b}	21.77±3.12^{c}	17.70±2.64^{e}	35.69±2.63^{c}	78.23±3.12^{b}	3.26±0.74^{b}	MR
T2	30.76±4.26^{c}	11.93±3.34^{b}	38.78±3.33^{a-b}	10.49±2.18^{b}	20.97±2.17^{b}	18.78±1.10^{f}	36.49±1.31^{d}	79.03±1.17^{b-c}	2.95±0.51^{a}	R
T3	30.21±2.41^{c}	13.41±2.57^{c}	44.42±2.13^{c}	10.40±3.30^{b}	20.81±2.61^{b}	16.30±1.52^{c-d}	38.40±2.34^{e-f}	79.19±1.61^{b-c}	2.93±0.72^{a}	R
T4	30.73±3.67^{c}	13.21±3.23^{c}	42.99±1.17^{bc}	10.08±2.72^{b}	20.15±1.45^{b}	18.01±2.75^{f}	36.51±1.59^{d}	79.85±1.45^{b-c}	3.07±1.04^{a-b}	MR
T5	28.82±1.28^{b}	13.49±1.66^{c}	46.86±2.74^{d}	11.15±1.78^{c}	22.30±1.57^{d}	17.66±3.84^{e}	37.54±1.00^{d-e}	77.70±1.57^{b}	3.01±0.53^{a-b}	MR
T6	25.13±4.38^{a}	11.75±2.24^{b}	46.80±5.08^{d}	9.09±1.41^{a-b}	18.18±1.44^{a-b}	11.89±1.41^{a-b}	37.80±0.96^{d-e}	81.82±2.83^{d}	2.82±0.44^{a}	R
T7	26.26±2.84^{a}	9.57±1.25^{a}	36.45±1.50^{a}	7.55±2.38^{a}	15.10±1.33^{a}	10.58±1.29^{a}	33.01±1.26^{b}	84.90±1.77^{e}	2.97±0.65^{a}	R
T8	30.32±2.52^{c}	13.57±2.38^{c}	44.80±2.66^{c}	10.44±3.31^{b}	20.87±0.77^{b}	16.52±1.00^{c-d}	34.18±2.11^{b-c}	79.13±2.75^{b-c}	3.31±0.54^{b}	MR
Mean	30.44±2.91	15.59±2.33	41.44±2.76	10.14±2.34	20.28±1.77	16.66±2.48	35.78±1.34	79.71±2.05	3.11±0.61	
	NOEL	NOAE	POEH	NOSD	POSD	POSWL	MDD	POBR	BSI	Status
Genotype Panna										
Untreated	34.97±1.84^{d-e}	14.41±0.52^{c-d}	41.36±1.65^{c-d}	11.37±0.55^{b-c}	22.73±1.11^{d-e}	19.98±2.07^{e}	34.61±0.45^{b-c}	77.27±1.11^{a}	3.35±0.07^{b-c}	MR
Control	34.33±1.15^{d-e}	14.27±1.09^{c-d}	41.56±2.18^{c-d}	10.13±2.06^{a-b}	20.27±2.12^{c-d}	29.70±3.25^{f-h}	30.08±1.07^{a}	78.99±2.12^{b-c}	3.84±0.01^{b-c}	MR
T1	30.38±2.03^{b-c}	11.91±2.33^{a-b}	39.21±2.15^{b-c}	10.30±1.13^{a-b}	20.60±1.07^{c-d}	19.89±2.75^{e}	36.51±2.13^{d-e}	79.29±2.07^{c}	2.95±0.03^{a}	R
T2	27.42±2.39^{a}	10.43±1.26^{a}	38.04±3.07^{a}	9.45±2.36^{a}	18.89±2.28^{a-b}	18.08±4.96^{c-d}	37.05±2.34^{f-g}	80.31±1.17^{d}	2.75±0.04^{a}	R
T3	31.18±1.56^{c}	12.37±2.09^{b}	39.67±2.19^{b-c}	9.79±1.09^{a}	19.58±1.18^{b}	14.89±3.25^{a}	37.45±1.15^{f-g}	81.23±1.18^{e-f}	2.92±0.01^{a}	R
T4	29.24±2.91^{b}	11.41±2.06^{a-b}	39.03±1.46^{b}	11.02±2.33^{b-c}	22.03±1.66	19.77±3.51^{e}	34.66±2.06^{b-c}	77.25±2.66^{a-b}	3.05±0.05^{b}	MR
T5	33.03±2.02^{d}	13.11±1.33^{b-c}	39.70±2.07^{b-c}	11.27±3.16^{b-c}	22.54±1.32^{d-e}	17.14±4.96^{c}	36.65±3.12^{d-e}	78.15±2.32^{b-c}	3.05±0.01^{b}	MR
T6	28.52±2.12^{a-b}	11.23±2.15^{a-b}	39.36±3.35^{b-c}	9.08±1.65^{a}	18.15±1.30^{a}	18.27±1.87^{c-d}	36.88±1.28^{d-e}	81.22±1.30^{e-f}	2.85±0.02^{a}	R

Continue table 3

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T7	27.20±1.76^{a}	10.66±3.24^{a}	39.22±2.99^{b-c}	9.36±0.72^{a}	18.71±1.40^{a}	15.27±1.23^{a-b}	36.79±1.53^{d-e}	81.51±1.45^{e-f}	2.79±0.13^{a}	R
T8	30.92±1.19^{b-c}	13.33±1.18^{b-c}	43.13±1.90^{e}	11.14±2.45^{b-c}	22.27±0.90^{d-e}	21.27±2.65^{e-f}	35.75±2.61^{c}	78.18±1.90^{b-c}	3.14±0.04^{b}	MR
Mean	32.61±1.89	13.49±1.73	42.25±2.30	10.77±1.75	21.53±1.43	20.63±3.05	35.19±1.77	78.42±1.72	3.19±0.05	
	NOEL	NOAE	POEH	NOSD	POSD	POSWL	MDD	POBR	BSI	Status
Genotype Howrah Local										
Untreated	73.89±1.06^{i-j}	55.66±1.47^{g}	75.33±1.44^{i-j}	34.59±0.84^{h}	69.17±1.68^{h}	67.51±1.18^{i}	21.08±0.38^{a}	30.83±1.68^{a-b}	8.29±0.15^{j}	S
Control	69.74±0.32^{h}	57.86±0.31^{f}	82.96±0.07^{j-k}	33.73±0.33^{g}	67.45±0.67^{g}	70.52±3.64^{j-k}	23.28±0.09^{a-b}	32.55±0.67^{a-c}	7.57±0.02^{i}	MS
T1	60.45±0.03^{e-g}	42.48±0.03^{f}	70.27±0.02^{h}	28.11±0.03^{e-f}	56.21±0.07^{e-f}	57.36±0.25^{h}	25.32±0.03^{a-c}	43.79±0.07^{f}	6.43±0.01^{g-h}	MS
T2	45.48±0.19^{b-c}	26.57±0.15^{c}	58.42±0.10^{d-e}	22.45±0.15^{c-d}	44.90±0.31^{c-d}	36.95±0.43^{c-d}	32.77±0.06^{d-f}	55.10±0.31^{h-i}	4.34±0.00^{c-d}	MR
T3	52.38±0.15^{f}	31.48±0.15^{e}	60.10±0.12^{f}	28.53±0.09^{e-f}	57.05±0.18^{e-f}	51.74±1.37^{g-h}	28.11±0.06^{d}	42.95±0.18^{d-e}	5.33±0.00^{f}	MR
T4	57.72±0.15^{e}	29.33±0.09^{d}	50.81±0.03^{d}	26.60±0.22^{e}	53.21±0.44^{e}	47.15±0.49^{f}	28.44±0.15^{d-e}	46.79±0.44^{e-g}	5.16±0.03^{e}	MR
T5	50.32±0.09^{c-d}	30.32±0.09^{e}	60.25±0.07^{f}	24.39±0.12^{d}	48.79±0.24^{d}	53.16±0.42^{h}	24.64±0.06^{b-c}	51.21±0.24^{g}	6.01±0.02^{g}	MR
T6	40.56±0.09^{a-b}	22.18±0.06^{b}	54.69±0.04^{d-e}	18.29±0.09^{b}	36.57±0.18^{b}	30.90±0.42^{a}	34.86±0.06^{e-h}	63.43±0.18^{j-l}	3.86±0.01^{a-c}	MR
T7	33.61±1.18^{a}	15.94±0.86^{a}	47.41±1.51^{a-b}	12.45±0.36^{a}	24.91±0.72^{a}	31.45±1.12^{a-b}	31.45±0.71^{e}	75.08±0.72^{m}	3.83±0.15^{a-b}	MR
T8	54.31±3.13^{d-e}	27.49±0.72^{c}	51.07±4.01^{b-c}	20.52±0.52^{c}	41.05±1.15^{c}	44.72±1.70^{e}	27.13±0.94^{b-d}	58.94±1.15^{j}	5.31±0.14^{e-f}	MR
Mean	51.61±1.18	31.51±2.25	59.55±2.08	23.89±1.17	47.79±2.34	47.10±2.41	28.44±0.72	52.20±2.34	5.31±0.22	
	NOEL	NOAE	POEH	NOSD	POSD	POSWL	MDD	POBR	BSI	Status
Genotype PDML-13-11										
Untreated	58.77±2.67^{i}	42.55±2.18^{h}	72.35±0.56^{i}	32.85±2.04^{g}	65.70±4.08^{g}	35.77±0.68^{e}	28.31±0.33^{a-b}	34.30±4.08^{a}	5.75±0.93^{f}	MR
Control	55.14±0.06^{h}	42.03±0.02^{g}	76.22±0.10^{j}	32.11±0.07^{g}	64.21±0.13^{g}	45.05±1.49^{g}	28.09±0.04^{a}	35.79±0.13^{a}	5.78±1.01^{f}	MR
T1	45.45±0.12^{e}	32.14±0.07^{f}	70.70±0.14^{g-h}	28.10±0.03^{f}	56.21±0.07^{f}	38.05±2.95^{f}	32.45±0.31^{d}	43.79±0.07^{b}	4.64±0.84^{d}	MR
T2	39.31±0.06^{c}	25.26±0.12^{c}	64.26±0.20^{e}	20.18±0.06^{d}	40.36±0.12^{d}	29.35±2.96^{c}	36.72±0.12^{e-f}	59.64±0.12^{d}	3.82±0.71^{c}	MR
T3	48.32±0.09^{f}	31.53±0.15^{e}	65.25±0.18^{e-f}	24.14±0.03^{e}	48.29±0.06^{e}	34.30±1.71^{c-d}	33.29±0.09^{d-e}	51.71±0.06^{c}	4.50±0.56^{d}	MR
T4	45.44±0.12^{e}	28.34±0.09^{d}	62.36±0.05^{d}	20.26±0.09^{d}	40.51±0.18^{d}	34.13±2.26^{c-d}	35.15±0.09^{e}	59.49±0.18^{d}	4.13±0.69^{d}	MR
T5	35.16±0.07^{b}	30.31±0.15^{d-e}	86.21±0.35^{j-l}	28.26±0.12^{f}	56.52±0.23^{f}	46.59±1.04^{g}	28.67±0.12^{a-b}	43.48±0.23^{b}	5.17±0.78^{e}	MR
T6	21.83±0.07^{a}	13.05±0.01^{a}	59.79±0.14^{c}	10.70±0.33^{a}	21.39±0.66^{a}	13.99±0.78^{a}	37.62±0.09^{f}	78.61±0.66^{g}	2.97±0.85^{a}	R
T7	41.89±1.20^{d}	16.68±2.12^{b}	39.82±2.73^{a}	13.91±0.75^{b}	27.83±1.50^{b}	22.35±6.82^{b}	34.88±1.12^{d}	72.16±1.50^{f}	3.51±0.64^{b}	MR
T8	51.99±0.94^{g}	25.36±1.22^{c}	48.84±2.09^{b}	17.25±0.88^{c}	34.50±1.77^{c}	33.10±1.62^{c}	31.41±0.93^{c}	65.50±1.77^{e}	4.47±0.59^{d}	MR
Mean	42.57±1.87	27.18±1.59	64.12±2.73	21.65±1.31	43.31±2.63	32.99±2.07	33.14±0.63	56.68±2.63	4.33±0.76	
	NOEL	NOAE	POEH	NOSD	POSD	POSWL	MDD	POBR	BSI	Status
Genotype HUM-16										
Untreated	44.03±1.15^{i}	22.42±2.47^{e-i}	50.94±2.40^{g-h}	15.11±2.56^{e}	30.21±1.12^{e}	28.20±1.26^{g}	26.38±0.62^{i-j}	69.79±1.12^{e-f}	5.13±1.14^{h-i}	MR
Control	42.08±2.03^{h}	21.06±2.44^{g-h}	50.05±1.08^{f}	14.48±2.15^{c}	28.95±1.81^{c}	29.84±0.55^{b-c}	31.84±0.86^{a}	71.51±2.81^{b}	5.07±0.95^{h}	MR
T1	36.30±2.12^{f}	18.53±2.09^{b-d}	51.04±2.06^{h-i}	14.13±3.03^{c}	28.27±2.29^{c}	23.71±1.32^{c-d}	26.10±1.05^{b}	71.73±3.29^{b}	4.39±1.01^{g}	MR
T2	31.49±2.22^{d}	14.12±1.32^{b}	44.83±1.64^{a-b}	12.53±2.15^{c}	25.05±2.06^{c}	21.13±1.46^{a-b}	29.21±2.59^{f}	74.95±2.06^{c}	3.22±0.88^{c}	MR
T3	38.26±1.09^{g}	18.28±2.12^{d}	47.79±3.19^{d}	13.31±3.12^{d}	26.61±1.29^{d}	19.19±1.18^{d}	36.24±1.36^{c}	73.39±1.29^{a}	4.07±0.72^{f}	MR
T4	30.45±1.12^{c-d}	14.35±3.07^{c}	47.14±3.34^{d}	13.12±3.36^{c}	26.24±2.24^{c}	24.84±1.44^{b-c}	31.35±2.29^{e}	73.76±2.24^{b}	3.42±1.05^{d}	MR

Continue table 3

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T5	29.29±1.72^{b-c}	13.68±1.12^{a-b}	46.71±2.20^{c}	12.24±2.12^{b}	24.48±1.12^{b}	20.97±2.22^{a}	35.28±1.46^{c}	75.52±3.12^{c}	3.56±1.01^{d}	MR
T6	25.61±3.20^{a}	12.04±2.09^{a}	47.01±3.07^{c-d}	11.76±2.31^{b}	23.51±2.23^{b}	21.61±1.29^{a}	32.47±2.12^{f}	76.49±1.23^{c}	2.90±0.69^{a}	MR
T7	27.89±2.36^{b}	13.56±1.25^{a}	48.61±6.45^{e-f}	9.78±1.87^{a}	19.55±1.74^{a}	18.87±1.69^{a}	34.75±1.66^{e}	80.45±1.74^{d}	3.13±1.11^{b}	R
T8	34.29±2.05^{e}	16.56±2.42^{d}	48.30±2.48^{e-f}	13.23±2.12^{d}	26.46±2.44^{d}	24.03±1.81^{a-b}	33.22±1.89^{d-e}	73.54±2.24	3.80±0.59^{e}	MR
Mean	32.85±1.90	16.46±2.03	48.24±2.79	12.97±2.48	25.93±1.83	23.24±1.42	32.27±1.40	74.07±2.21	3.87±0.91	

Note: Mean shows with standard error (±) of three replications. In the column, means followed by the different letters are showed significantly different at P>0.05 through Duncan's multiple range test (DMRT).

Note: NOEL: Number of egg laid, NOAE: Number of adult emerge, POEH: Percentage of egg hatched, NOSD: Number of seed damage, POSD: Percentage of seed damage, POSWL: Percentage of seed weight loss, MDD: Mean development days, POBR: Percentage of bruchid resistance, SI: Susceptible index, R: Resistant, MR: Moderately Resistant, MS: Moderately susceptible, S: Susceptible.

phenolic content. Radha and Susheela (2014) reported cowpea seeds treated with neem kernel seeds where phytochemicals could interfere and control ecdysone synthesis in bruchid. Therefore, it could be hypothesized that some of the phenolic compounds might increase the production of allatostatins. Allatostatins is a neuropeptide inhibitor which inhibits the biosynthesis of JHs (juvenile hormones) in neuroendocrine glands. Allatostatin might inhibit the process of Vitellogenesis that is why deposited fewer number eggs and reduced metamorphosis. The seeds of fenugreek (T3) show the most effective result on genotype Panna and identified as bruchid resistant. Nevertheless, this treatment reduced the number of seed damage in other five genotypes too. One obvious interpretation is that the feeding assay of bruchids might have been suppressed severely by the bitterness of fenugreek seeds (T3). The adult bruchid could perceive the bitterness through their taste organ during oviposition on the treated seeds in selected mungbean genotypes. Although female bruchid deposit fewer number eggs on treated seeds, the larvae might be rejected the seeds for penetration and could not survive due to feeding inhibition. That is why the bruchid could not complete their life cycle and reduced the chances of seed damage for T3. In the assay of antifeedants, the feeding process is suppressed by chemical components which affect the central nervous system (CNS) of insect is reported by Isman *et al*. (1990). Allicins, a chemical compound found in ginger as well as other plant materials were responsible for reducing the population of aphids in bean (*Phaseolus vulgaris*) seeds (Tegegne, 2017). The longest mean development days (MDD-38.40) is observed in genotype Pusa Baisakhi through the treatment of fenugreek seeds (T3) among all the treatments in selected genotypes. The longer and shorter MDD should change the period of the bruchid life cycle. Singh (2011) reported that a longer developmental period of 35.00 days was recorded in cowpea treated with mahogany bark compared to control (30.16 days). The peel of sweet orange (T4) is found effective on genotype SML-302 and Pusa Baisakhi and are presented as bruchid resistant. After treatment of sweet orange fruit peel (T4), bruchid oviposition was decreased and consequently the number of seed damaged was reduced. It might be due to the action of essential oil which might be generated toxicity effect on the first stage of larva and for that the larva could not penetrate the seed coat. Singh (2011) stated that the presence of aromatic properties in citrus leaf peel is the cause of deterring the bruchid oviposition. Although the chickpea seeds treated by the citrus peel showed almost same number of egg deposition in control, clear result of preventing the bruchid attack is reported by Elhag (2000). This observation supports our result. In ancient Egypt, black cumin seeds were used as preservative during mummification. The seeds of black cumin (T6) shows most effective treatment against the bruchid attack and thus in preventing the seed damage. This is supported by their low bruchid susceptibility indices in genotypes SML-302, Pusa

Baisakhi, Panna, PDML-13-11 and HUM-16 except Howrah local. Thymoquinone is the main constituent in black cumin seeds and could have the synergistic properties which might drastically prevent the seed damage from bruchid attack. It could be predicted that Thymoquinone might activate the elicitor molecules situated on the cell membrane of mungbean seeds and in turn trigger the IDS (intracellular defense signaling) through the pathway of octadecanoid. For that, it could enhance the synthesis of metabolites which ultimately increases the ability of seed resistance and reduced the bruchid attack. Cowpea seeds treated with the dust of cloves (*Syzygium aromaticum*) exhibited significantly less egg deposition by bruchid compare to control was reported by Oparaeke and Daria (2005). *Thuja occidentalis* is known to act on blood, gastrointestinal tract, kidney and brain (Borricke, 2000). In this study, Thuja 30 CH (T7) inhibited maximum number (36.45) of egg hatched in genotype Pusa Baisakhi and thus caused the deterrence of seed weight loss (6.59%) in genotype SML-302. This proves to be an effective treatment of bruchid management. Among the six genotypes, Panna and HUM-16 appeared to be bruchid resistant through the treatment of Thuja 30 CH. This result reveals that Thuja 30 CH was triggering the inherent self-defense mechanism through the vital force of the plant which might be developing the capability of pest-resistance. The globules of Thuja 30 used in chickpea to control root rot fungi was found to prevent the colonization of root rot and also increased the plant growth as reported by Hanif and Dawar (2016). During the preparation of *Arsenicum album*, Hahnemann (1810) noticed that the dilution factor was high enough to consider the final solution having almost not a single molecule compare to the stock (Arsenious acid or

Table 4: Compare of the different treatment effects against different parameters of bruchid attack across the mungbean genotypes.

	NOEL	NOAE	POEH	NOSD	POSD	POSWL	MDD	POBR	SI
Untreated	45.87^{l-o}	27.88^{i-k}	57.65^{i-o}	21.91^{i-m}	39.69^{k-p}	32.95^{h-l}	28.56^{a-c}	60.31^{a-d}	5.07^{e-i}
Control	43.61^{j-m}	27.33^{i-k}	57.67^{i-o}	18.95^{f-j}	37.89^{j-o}	36.38^{j-o}	29.36^{a-d}	62.06^{b-e}	4.99^{d-i}
T1	32.83^{b-e}	22.14^{g-h}	53.79^{h-m}	17.19^{e-j}	34.38^{h-l}	29.04^{f-i}	31.98^{b-d}	65.59^{c-g}	4.13^{c-h}
T2	33.18^{b-f}	16.44^{b-e}	47.70^{e-i}	13.88^{a-c}	27.76^{d-g}	20.25^{b-d}	34.77^{d-g}	72.11^{i-n}	3.31^{a-c}
T3	38.47^{e-i}	19.87^{e-f}	49.52^{f-j}	16.11$^{c—h}$	32.23^{g-i}	25.09^{c-f}	34.56^{c-g}	67.91^{g-i}	3.81^{b-e}
T4	36.86^{e-h}	17.95^{c-e}	47.03^{e-g}	15.07^{c-g}	30.13^{f-i}	25.42^{c-f}	33.51^{c-f}	69.75^{h-l}	3.64^{b-d}
T5	34.33^{e-g}	18.92^{d-f}	53.81^{h-m}	16.11^{d-g}	32.86^{g-i}	29.03^{f-i}	32.52^{b-e}	67.25^{d-i}	4.03^{c-h}
T6	28.42^{a-c}	13.64^{a-c}	48.45^{f-i}	11.45^{a-b}	22.89^{b-e}	17.28^{a-c}	35.99^{e-i}	77.01^{k-p}	3.25^{a-c}
T7	30.30^{a-c}	12.95^{a-b}	42.74^{a-d}	14.11^{b-e}	20.63^{a-c}	17.52^{a-c}	34.28^{c-g}	79.40^{l-s}	3.21^{a-c}
T8	43.39^{i-l}	18.25^{d-f}	47.32^{e-h}	13.81^{a-c}	27.78^{d-g}	25.56^{c-f}	34.77^{d-g}	72.29^{i-n}	3.89^{b-e}

In the column, means followed by the different letters are showed significantly different at P>0.05 through Duncan's multiple range test (DMRT).

Note: NOEL: Number of egg laid, NOAE-Number of adult emerge, POEH: Percentage of egg hatched, NOSD: Number of seed damage, POSD: Percentage of seed damage, POSWL: Percentage of seed weight loss, MDD: Mean development days, POBR: Percentage of Bruchid resistance, SI: Susceptible index, R: Resistant, MR: Moderately Resistant, MS: Moderately susceptible, S: Susceptible.

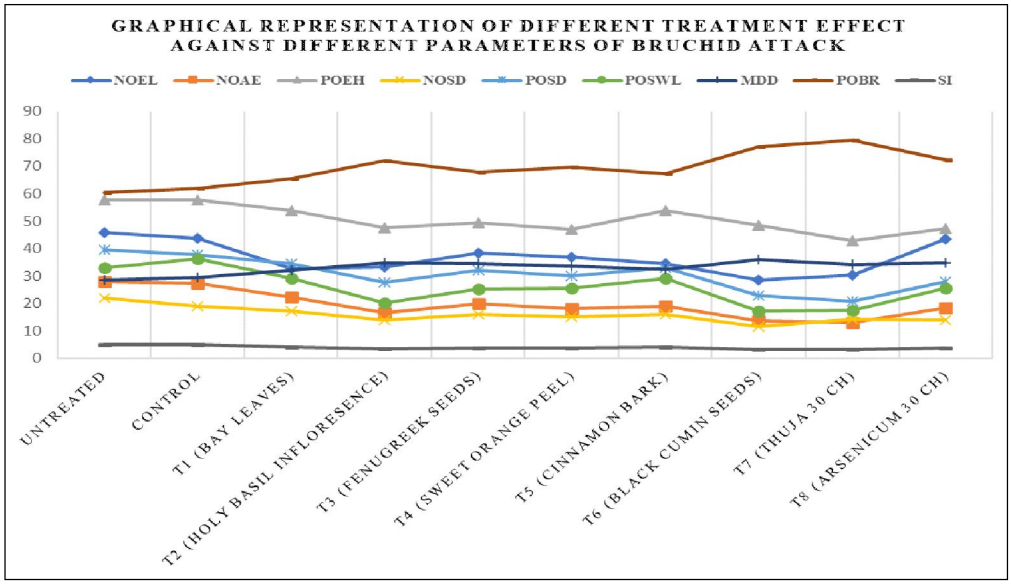

Fig 1: Effect of natural eco-friendly plant materials and homeopathic dilutions on different parameters of bruchid attack.

Arsenic trioxide). However, it was found effective remedies of different diseases in every organ. The homeopathic dilution Arsenicum 30 CH (T8) and Cinnamon (T5) also reduced the oviposition, seed damage, seed weight loss and increase the seed resistance percentage and showed the protecting ability but could not show any potential to impart resistance on any genotypes. Jana (2000) applied different dilution of *Arsenicum album* on jute plant and recorded the significantly better result of all morphological traits and showed the crop improvement. From this result, it could be established that the homeopathic dilution exhibited their efficacy to bruchid management. In Bruchid management, the homeopathic dilution could be proposed as a 'Homeo-Bruchid Interaction'.

Among all the treatment, the inflorescence of holy basil (T2-*Ocimum sanctum*) and seeds of black cumin (T6 - *Nigella sativa*) revealed the most effective treatment for bruchid control in most of the selected mungbean genotypes. The present study confirms that the treatment materials chosen instinctively have a broad spectrum of potentiality to bruchid management in mungbean.

CONCLUSION

This study reveals that a wide range of seed treatment materials can be chosen from nature that could effectively manage the pest control, in particular bruchid control of mungbean storage. It is important for both economic issues and health issues. Based on earlier similar studies of pest control management on other grains, we have instinctively chosen our eight treatment materials. All of these are eco-friendly, easily available and much less expensive. Except the fruit peel of sweet orange, none of the other seven treatment materials were tested before. Therefore, it was not known to any degree whether they have any ability to deter the bruchid infestation. Therefore, this study highlights a new promising eco-friendly approach for bruchid management either through the 'Homeo-Bruchid Interaction' or through the 'Easily Available Homemade Remedies.' The farmers could be easily applying to this non-expensive management strategy as a supplement of chemical pesticides to protect the store product from bruchid infestation. It is necessary to further investigate properly in assessing the quality components of treating seed as well as to identify the particular properties which were responsible for bruchid resistance and also confirmed the level of human safety based on their bioactivity before commercialization.

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Conflict of interest

The authors declared that they do not have conflict of interest.

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