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RESEARCH ARTICLE

Local treatments and vacuum sealing as novel control strategies for stored seed pests in the tropics

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Abstract Prevention of pests while maintaining viable seed during storage is often challenging for smallholder farmers in the tropics and subtropics. Investment in costly technologies or storage equipment is often unavailable or economically unreasonable, and alternative methods of seed storage can play a role in ensuring regional and global food security. This research evaluates whether or not vacuum sealing and locally available seed storage treatments are effective techniques to control cowpea bruchid (*Callosobruchus maculatus*). This research also assesses the effects of such techniques on the viability of stored Lablab (*Lablab purpureus* L.) seed in the humid tropics. Tested treatments included vegetable oil, pulverized bamboo charcoal, galangal powder, powdered detergent, a bleach solution, and carbaryl. Infested seed samples stored in northern Thailand under local treatment options and vacuum sealing were evaluated between May 2011 and May 2012 for bruchid presence, seed viability, and seed vigor. After 1 year of vacuum storage, seed viability was 77.6% compared with 66.5% under non-vacuum conditions. Over that period, vacuum storage successfully prevented bruchid population growth (4.9 compared with 123.3 insects per 50 seeds under non-vacuum conditions; $F = 22.59$, $P < 0.001$). By contrast, the oil treatment greatly reduced seed viability (1.3%), although it restrained bruchid population growth (3.5 compared to 97.0 insects per 50 seeds). Other local treatments (galangal powder, carbaryl, and bamboo charcoal) limited bruchid population growth ($F = 8.37$, $P < 0.05$) compared with the control, while maintaining seed viability. Seed germination duration was not affected by vacuum sealing and seed treatments but was rather influenced by changing environmental conditions throughout the trial. These seasonal changes also influenced overall insect lifecycle and seed metabolism. These results demonstrate that vacuum sealing and several locally available treatments provide novel, low-cost, appropriate seed storage options for local seed banks and smallholder farmers in the developing world, thus avoiding the use of locally rare or expensive chemicals, low temperature, or low moisture conditions.

Keywords Bruchids · Vacuum sealing · Lablab · Stored seed pests · Seed storage treatments · Crop biodiversity · Germplasm

1 Introduction

Storing seeds effectively in subtropical and tropical locations is often complicated by insect damage during storage (Upadhyay and Ahmad 2011). Consistent high temperatures and humidity increase the rate and development of insect pests and also increase dormant seed metabolism (Lale and Vidal 2003), reducing seed viability. Although much storage research has been conducted to reduce insect damage and ensure quality seed in temperate regions, where few of these problems are continuous due to seasonality, little work has been done to determine the best storage conditions for seed in the tropics (Rao et al. 2006; Croft et al. 2012). More significantly, minimum research has focused on solutions for small-scale, small-lot seed saving for subsistence farmers and community seed banks that could prevent unnecessary losses from insect damage of harvested seed (Croft et al. 2012). Identifying appropriate seed storage conditions that discourage granivorous

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insects in these contexts could increase farmers' livelihoods through greater food security and allow a wider audience of farmers and small seed banks to affordably store and improve cultivated seed quality and diversity (Chauhan and Ghaffar 2002).

ECHO Asia, in Mae Ai, Thailand, maintains a small-scale seed bank to distribute sample-sized packets of meritorious seeds to development workers, organizations, and farmers and exists as an educational platform to promote seed saving, diversity, and appropriate seed technologies (Croft et al. 2012). ECHO seed bank staff first noticed insect damage within stored seed at the ECHO seed bank in 2011. The insects observed were cowpea bruchids (*Callosobruchus maculatus*; Ahn et al. 2013) within *Bruchinae*, a subfamily of *Chrysomelidae* (Appert 1987), which are common in farmers' fields around the seed bank and widely distributed throughout the tropics and subtropics (Bailey 2007) (Fig. 1).

Cowpea bruchids lay eggs directly on seeds in the field, which later hatch in storage, making prevention and detection difficult (Appert 1987; Chauhan and Ghaffar 2002). From the egg location, larvae enter the seed, consuming the endosperm as food, leading to substantial losses in grain weight and endosperm reserves (Blumer and Beck 2010). Developing pupae inside the seed leave behind a translucent film on the seed coat referred to as a "window." Fully developed adults emerge through the windows leaving holes (Singh et al. 1985) and continue reproducing and laying eggs on nearby seeds. The time required for the bruchid lifecycle decreases with increasing temperature (reaching an optimum between 30 and 35 °C), and more adults successfully emerge from seeds at higher relative humidity (Lale and Vidal 2003). High seed moisture content also increases activity and reproduction levels (Upadhyay and Ahmad 2011). Fecundity in females appears to be positively correlated with temperature and relative humidity, as both factors individually increase the number of eggs deposited (Lale and Vidal 2003; Ouedraogo *et al*. 1996). Because higher temperature and relative humidity enable bruchids to complete one lifecycle often in less than 30 days (Lale and Vidal 2003; Singh et al. 1985), bruchid populations have the ability to rapidly increase if seeds are stored for many months without proper storage conditions in the tropics (Ouedraogo *et al*. 1996; Chauhan and Ghaffar 2002).

Analogous to all insects, bruchids cannot develop or complete their entire life cycle without oxygen (Motis 2011; Appert 1987). Studies on cowpea bruchid metabolism and resistance to low-oxygen environments suggest that late-stage larva and adults stop development in 2–6% oxygen environments yet note some early instar larval stage resistance, capable of surviving in hypoxia up to 2 weeks (Ahn et al. 2013). Oxygen consumption of rapidly growing bruchid populations within sealed containers may be enough to reduce and possibly retard their own growth (Ahn et al. 2013), yet loss of seed weight occurring prior to this self-created hypoxia would be detrimental to the farmer. Vacuum sealing of stored seed has proven to be effective in preventing insect growth in previous studies (Van Huis 1991; Croft et al. 2012; Chiu *et al*.

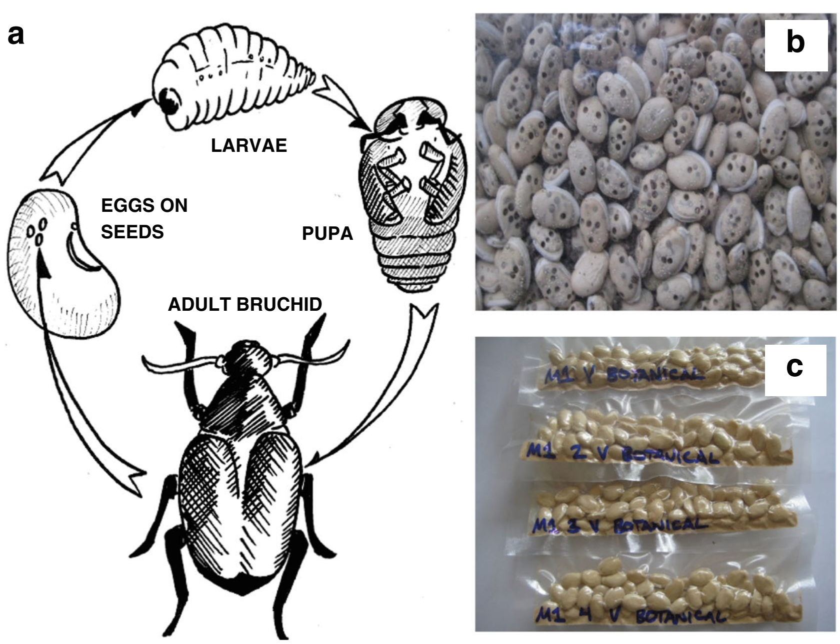

Fig. 1 Cowpea bruchids, *Callosobruchus maculatus*, found in smallholder agricultural fields throughout the tropics and subtropics of the world, are a common pest within stored seeds. In warm and humid conditions, bruchid populations rapidly increase due to their lifecycle (**a**), leading to substantial losses in lablab (*Lablab purpureus* L.) seed weight, which limits food supply and viable planting material for farmers (**b**). This research examined the novel use of vacuum sealing and local treatments for the successful control of bruchids in stored lablab seed applications (**c**)

2003) and has been shown to maintain viability of stored seed in the humid tropics (Croft *et al*. 2012). Utilization of commercial vacuum sealers, common throughout Southeast Asia to package food, may have merit for small seed banks in the tropics for seed storage. The use of vacuum sealing as a means of insect control (by creating a low-oxygen environment) while maintaining seed viability (by prohibiting seed moisture uptake) may provide simultaneous appropriate solutions to several common storage issues for local seed banks.

Alternatively to vacuum sealing, zeolite beads are an emerging technology and have been promoted for the drying, storage, and prevention of pest predation of seeds (HIL 2015). However, the beads can be difficult for farmers to source locally in Thailand and are relatively expensive ($10.00–20.00 per kg) compared to local storage technologies explored in this study. In addition, there is some concern that over- or under-drying of seeds, requiring a thorough understanding of seed drying curves which may be out of reach of regional farmers, can be detrimental to seed viability (Hay et al. 2012). Whereas the principal function of zeolite is to reduce seed moisture content (HIL 2015), vacuum sealing offers the benefit of both creating a low-oxygen environment and maintaining low seed moisture content, conditions which are both beneficial to seed storage and stored seed pest control and may benefit local seed banks (Croft *et al*. 2012).

Because technologies like vacuum sealing and zeolite beads are not always available to the small-scale farmer, research is needed to better understand the efficacy of locally sourced and naturally occurring pesticides with or without vacuum sealing. Previous research suggests that abiotic, physical, and/or chemical properties of substances added to stored seed can be effective at controlling insects in other parts of the world (Chauhan and Gaffer 2002; Manzoomi et al. 2010; Songa and Rono 1998; Upadhyay and Ahmad 2011; Van Huis 1991), but opportunities exist to identify low-cost, locally available seed treatments which may aid smallholders. Along with ease of local acquisition, naturally occurring pesticides can be low-cost, biodegradable, and environmentally friendly for both farmers and stored seed compared to synthetic pesticides (Amuji et al. 2012; Van Huis 1991). Inert substances like diatomaceous earth, ash, sand, and charcoal have all shown to reduce insects through abrasion and desiccation in the stored seeds of other legumes (Almekinders and Louwaars 1999; Appert 1987; Upadhyay and Ahmad 2011), while various volatile oils including neem (*Azadirachta indica* A. Juss.), *Artemisia sp*., and cloves (*Syzygium aromaticum* (L.) Merrill & Perry) have reduced bruchid populations (Upadhyay and Ahmad 2011; Van Huis 1991). Aqueous extracts of ginger were effectively used to control bruchid on the seeds of cowpea in Nigeria (Amuji et al. 2012) and reduced oviposition rates (Upadhyay and Ahmad 2011). Solar radiation prior to storage eradicated bruchids in Kenya (Songa and Rono 1998) and solar heating in clear plastic proved successful in India (Chauhan and Gaffar 2002). Other high temperature (55–65 °C) treatments successfully controlled insects on stored grain (Upadhyay and Ahmad 2011), while storage under low temperatures (<12 °C) prevented insect growth while maintaining seed viability (Croft et al. 2012; Upadhyay and Ahmad 2011). Other physical barriers, such as retaining leguminous pods around seeds have had some success preventing bruchid damage along with the use of black-lights, corn oil, smoke, insect pheromones, and many other essential plant oils (Chomchalow 2003; Manzoomi et al. 2010; Songa and Rono 1998; Upadhyay and Ahmad 2011; Van Huis 1991). Aggregation of these naturally occurring pesticides and pest deterrents suggests that smallholder farmers could have access to more feasible, less-expensive solutions than conventional synthetic pesticides used during seed storage. Exploring available seed treatments in northern Thailand that have been recommended by regional farmers and have not previously been evaluated, in conjunction with vacuum sealing, may reduce critical gaps in knowledge of best seed storage practices readily available in the region at both the farmer and seed bank scales.

As part of the ongoing endeavors of ECHO, Inc. to promote world-wide food security for the poor and smallholder farmers, this study continues the exploration of options accessible at both the local, organizational, and community levels for storing and preserving seed germplasm in the tropics. The combination of vacuum sealing and locally available low-cost seed treatments in the tropics are explored in this study. The efficacy of vacuum sealing for simultaneously improving stored seed viability and reducing insect pests has implications for other facilities or institutions like the ECHO Asia Seed Bank at the non-governmental organization (NGO) level wishing to collect, maintain, and distribute seeds without relying on conventional and expensive methods of storage in the tropics (Croft et al. 2012).

Researching the effectiveness of vacuum sealing and locally available seed treatments that have not previously been studied can improve understanding of short-term seed storage biology and granivorous pest physiology at the village or local seed bank level between growing seasons. The purpose of this research was to evaluate the validity of vacuum sealing and locally available seed treatments (vegetable oil, pulverized bamboo charcoal, galangal powder, powdered detergent, a bleach solution, and carbaryl) for their control of cowpea bruchid (*C. maculatus*) populations and their effect on the viability of stored Lablab (*Lablab purpureus* L.) seed in the humid tropics in order to address cost and resource constraints for small-scale farmers and small seed banks in many underdeveloped locations. This research is novel because it explores the simultaneous application of vacuum sealing with local seed treatments as means to reduce storage pests while maintaining seed viability. The combination of these methods may provide novel options for seed savers in resource-constrained settings within the humid tropics and subtropics.

2 Materials and methods

2.1 Location and seed

The study took place at the ECHO Asia Seed Bank, 121 M. 8, Tambon Mae Namwan, Ampur Mae Ai, Chiang Mai, Thailand 50280 (20°1′N, 99°17′E) between 27 May 2011 and 25 May 2012. Storage conditions within the ECHO Asia Seed Bank cold room were maintained at 16 °C within an insulated room using an air conditioner controlled by a CoolBot™, but ambient temperature, relative humidity, and light all fluctuate seasonally, with lowest temperatures recorded in November through January and reduced precipitation and relative humidity during the same timeframe (Fig. 4a, b). A bruchid-infested supply of white-seeded lablab was purchased from a local farmer in Chiang Dao, Thailand, 1 month before the trial began. Lablab (*L. purpureus* L.), an orthodox seed used extensively as a cover crop and fresh pod vegetable by smallholders in the Southeast Asia region, is often observed with bruchid presence and damage. Consistent regional farmer demand for lablab suggested its appropriateness to be studied and to serve as a model for other stored seed commodities (e.g., cover crops, oil seeds, and seeds of food crops), which are prone to stored seed pests. Relatively large-sized lablab seed also allowed for easier identification of bruchid presence as opposed to smaller size legume seed.

Seeds were cleaned and sorted to remove seeds with any physical damage, insect damage, or mold prior to the trial, while seeds with visible signs of bruchid eggs were retained. The average number of bruchid eggs on seed coat exteriors was 1.75 eggs per lot of 50 seeds. The study was preceded by a baseline germination test of 970 seeds on 16 May 2011 and was determined to be 86% using the same germination protocol used throughout the duration of the experiment. Additional germination tests showed that seeds with visible bruchid damage were still able to germinate.

2.2 Experimental design

The trial was established to study the effects of vacuum sealing and seven seed treatments on (1) total bruchid presence (pest load), (2) seed germination rate (seed viability), and (3) mean time to 50% germination (seed vigor). The study was conducted as a factorial in a randomized complete block design (RCBD) with four replications. Main effects consisted of vacuum sealing treatments (vacuum sealed and not vacuum sealed) and local seed treatments (control, 10% bleach solution, galangal powder, carbaryl powder, pulverized bamboo charcoal, laundry detergent, and vegetable oil) for a factorial of 14 treatments. A total of 14,000 seeds divided into 280 individual lots of 50 *Lablab sp.* seeds (4 replications of 7 seed treatments by the factorial of 2 vacuum sealing treatments, for a total of 56 experimental units for each of the 5 sampling months (Month 1—M1, Month 3—M3, Month 6—M6, Month 9—M9, and Month 12—M12) not including the baseline germination trial) were prepared according to their predetermined randomized treatment on May 27, 2011 and placed in cold room storage. At each sampling month starting the first month (M1), seed lots were withdrawn and analyzed for bruchid presence, seed viability, and seed vigor at 3-month intervals: August 18 (M3) and November 29, 2011 (M6) and February 24 (M9) and and May 25, 2012 (M12).

2.3 Treatments

2.3.1 Vacuum sealing treatments

Half of the seed experimental units were stored under the main effect of vacuum sealing (0.080 MPa) (DZ-320A Vacuum Packing Machine, Hongzhan, China; 4-kg capacity; $530) in clear vacuum seal bags, while non-vacuum-sealed seed experimental units were double bagged in both a Ziploc™ quart-sized bag and one clear vacuum bag after the first month (M1) to prevent bruchids eating through the Ziploc™ bag. Non-vacuum-sealed samples were opened once a month to expose them to fresh oxygen.

2.3.2 Local seed treatments

Locally available seed treatments used in this study were readily available to the smallholder and reaffirmed through previous research along with observations and discussions with local residents around the ECHO Asia seed bank. A 10% by volume bleach solution (Haiter™ Brand; $1.16 per L), already employed regularly by the seed bank to disinfect seeds prior to germination testing, was included as an aseptic, low-input chemical technique. Seeds assigned to this treatment were washed with the bleach solution for 4 min followed by four consecutive rinses with sterile water before being allowed to dry and then assigned to their vacuum sealing treatment. Strong oxidation reactions of bleach could possibly penetrate existing bruchid eggs and windows on seeds and quickly kill developing insects. Powder from galangal (*Alpinia galanga* (L.) Willd.), a ginger relative purchased from Yok market in Chiang Mai ($8.72 per kg), was used as botanical chemical control agent. The ginger family (Zingiberaceae) contains several phenolic compounds including eugenol that demonstrate toxicity to insects (Chomchalow 2003). Carbaryl, in the form of the commercial brand 'Sevin 85' (Bayer™; $11.11 per kg) was diluted to a 10% mixture with baby powder (Johnson and Johnson™; $3.95 per kg) and was included for its insecticidal properties. The insecticidal nature of several enzyme inhibitors, which have been realized since their introduction in the 1950s (Bayer Environmental Science 2003), allowed the carbaryl to act as a chemical "control" that could provide a gauge by which to compare the effectiveness of the other treatment substances. Charcoal, made from locally pulverized bamboo (free, but also available commercially for \$0.70 per kg) was included as a possible inert substance that could desiccate larval stages of insects (Almekinders and Louwaars 1999; Songa and Rono 1998; Van Huis 1991). Detergent powder (locally purchased 'Breeze'; \$1.84 per kg) was observed within the regional community to protect seeds. Soaps and detergents act as denaturing agents to exposed cell membranes of soft-bodied insects. Finally, a vegetable oil (\$1.39 per L) was included because previous research has shown oil inhibits insect respiration (Van Huis 1991). Each of the treatments, not including bleach, was combined with seeds at a ratio of 0.5-mL (1/8 tsp) treatment per 50 seeds in each seed lot.

2.4 Determining total bruchid pest load

During each sampling month, individual samples (experimental units) of seeds were removed from the seed storage room and were analyzed for bruchid infestation. Bruchid eggs found on seeds, emergence holes on seeds, windows on seeds, and adult bruchids were all individually counted and summed to calculate the total pest load for each sample.

2.5 Determining seed viability and vigor

After the total bruchid pest load data was collected, seed viability was determined according to protocol methods described by Rao et al. (2006). During each sampling month, a randomized complete block design of four replications of 50 seeds from each sample were plated on petri dishes and placed in a temperature-controlled seed germination chamber set to maintain 28 ± 5 °C heated by two 10-W fluorescent light bulbs. The use of blocking in the chamber helped to minimize variation. Every other day for 14 days, germinated seeds (as determined by radical emergence from the embryo (Rao *et al.* 2006)) were removed and counted to calculate the total viability. Seed vigor, represented as mean time to 50% germination, was also calculated from data collected over the 14 days (Croft et al. 2012). Seeds showing bruchid emergence holes were counted separately from undamaged seeds to determine if viability of seeds with bruchid damage was statistically different than those without damage. Additional determinations of seed viability during each sampling month were conducted using the same protocol.

2.6 Data analysis

To determine differences between vacuum sealing and local seed treatments across and at each sampling period, all data were analyzed with analysis of variance (ANOVA) and Fisher's least significant difference (LSD) post hoc tests using the mixed procedure of SAS and the PDMIX800 macro (Saxton 1998). For all dependent variables, degrees of freedom were adjusted using the Satterthwaite correction, and normality of the raw data and residuals was evaluated using the UNIVARIATE procedure of SAS. Excluding extreme outliers within sampling months and using a Log10 transformation normalized total pest load data. Because of the extreme influence of the oil treatment on overall seed germination rate, oil treatments were excluded to analyze differences within the main effect of vacuum sealing. Although ANOVA included interaction effects, we limited our inference space to the main effects of vacuum sealing and local seed treatments at each sampling period in order to assist the ECHO Asia Impact Center to discern best practices for seed storage treatments and create baseline data for bruchid population growth and seed viability decomposition over time. Sampling date was regressed with mean seed germination rate and mean time to 50% germination for vacuum sealing treatments and local seed treatments using the linear or quadratic function of the REG Procedure of SAS.

3 Results and discussion

3.1 Total pest load

Total number of bruchid eggs, windows, emergence holes, and adults, represented as the total pest load within each sample, was most influenced by the main effect of vacuum sealing and resulted in significant differences between vacuum-sealed and non-vacuum-sealed treatments ($F = 22.59$, $P < 0.001$) from the first month (M1) onward (Fig. 2a).

Removing air from samples through vacuum sealing resulted in very few bruchids reaching maturity and greatly limited the total predation of seeds. Although larvae have some survival capability in short periods of hypoxic environments (Ahn et al. 2013), vacuum-sealed samples remained free of large bruchid populations from the beginning of the experiment. For stored insect pest protection alone, vacuum sealing provides a low-cost alternative to other methods of seed storage (Croft et al. 2012). Based on available resources, both the smallholder and small organizations could dramatically reduce pest predation on stored seed using vacuum sealing.

The exponential increase of bruchids within non-vacuum-sealed samples across treatments reveals stored seed is subject to high levels of predation (Fig. 2a). The decrease in average pest load during the final sample month appears to be the result of a time-lag effect following several months of cooler, drier conditions prior to the sampling time. The lower temperature and humidity during these months could have decreased reproduction and success of adult bruchid emergence from seed (Lale and Vidal 2003; Upadhyay and Ahmad 2011). Non-vacuum-sealed bags were exposed each month to oxygen and repeated introductions of lower humidity during the 3 months leading up to the final sampling period (M12),

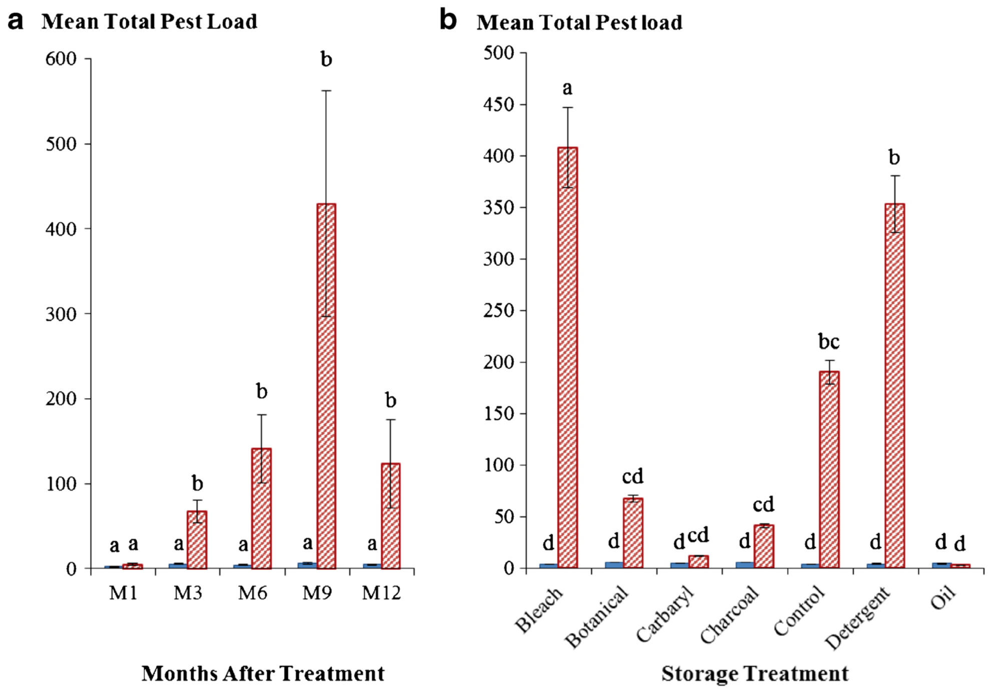

Fig. 2 The effect of **a** vacuum-sealed (*blue*) and non-vacuum-sealed (*red stripes*) seed treatments on total pest load averaged across local seed treatments by sampling month and **b** mean total pest load in vacuum-sealed (*blue*) and non-vacuum-sealed (*red stripes*) samples by locally available seed storage treatment averaged across 1 year of storage. Mean separation *letters* above columns represent statistically significant differences at alpha = 0.05. *Error bars* represent ± standard error of the mean. In **a**, total pest load increases in non-vacuum-sealed samples following the first month (M1), while vacuum sealing effectively limits bruchid populations across the experiment. Vacuum sealing consistently limits bruchid population regardless of individual treatment in **b**, while botanical, carbaryl, charcoal, and oil treatments all reduce bruchid numbers

which could lead to changes in bruchid growth. During months of high humidity, rainfall, and temperatures, bruchids rapidly grew in population within non-vacuum-sealed samples. Even though non-vacuum-sealed samples were enclosed in two bags, the influence of monthly oxygen exposure on bruchid population was beneficial (Ahn et al. 2013; Motis 2011). Future analysis of bruchid growth over several years could separate the influences of seasonal climate conditions from oxygen exposure and determine which has a greater effect on bruchid populations in stored seed over longer periods of time. However, since the critical storage time for many smallholders in subtropical areas is between growing seasons, short-term and low-cost-efficient storage methods should be further researched in addition to answering long-term storage questions of seed health under vacuum storage.

The total pest load as influenced by local seed storage treatments across the trial was significantly different ($F = 8.37$, $P < 0.05$). Several treatments, including botanical (galangal powder), carbaryl, charcoal, and oil, all had reduced total pest loads in non-vacuum-sealed samples when compared to the detergent and bleach treatments, but all contained higher pest loads compared to their vacuum-sealed counterparts (Fig. 2b). The most effective of the treatments was vegetable oil, which effectively limited bruchid population in both the vacuum and non-vacuum-sealed containers (Fig. 3b). However, the oil treatment also reduced seed viability, and thus is only recommended when storing seeds for later consumption. Further studies could analyze a range of oils that inhibit insect respiration (Van Huis 1991) while not interfering with the respiration of dormant seeds. Although not significantly different than the control ($P > 0.05$), the botanical (galangal powder), carbaryl, and charcoal treatments all showed promise in reducing the total population of bruchids. Moreover, the carbaryl treatment effectively controlled bruchid populations in both vacuum and non-vacuum sealed samples, suggesting its application for the smallholder. Both the bleach and detergent treatments had no effect on bruchid population in non-vacuum-sealed samples, and the population of bruchids within the bleach treatment was significantly higher ($P < 0.05$) than the control. Why the bleach treatment caused an inflated population when compared to the control is unknown but could be explained by the bleach chemical oxidizing and softening the seed coats, making them more vulnerable to predation. In a similar way, although not statistically different ($P > 0.05$) than the control, the increase in bruchid predation on seeds within the detergent treatment could be explained by action of the detergent reducing wax layers on seed coats.

In addition to the chemical properties of the treatments themselves, physical distribution of treatment chemicals

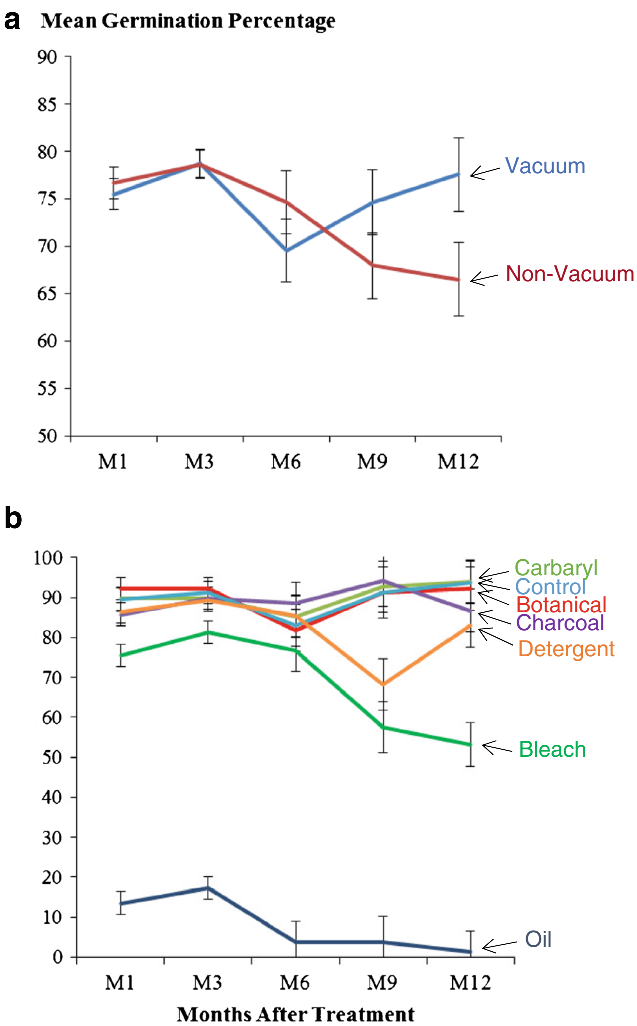

Fig. 3 Mean seed germination percentage for **a** vacuum sealed (*blue*) and non-vacuum sealed (*red*) seed across locally sourced seed storage treatments by sampling month along with **b** mean seed germination percentage for local seed storage treatments: bleach (*dark green*), botanical (*bright red*), carbaryl (*light green*), charcoal (*purple*), control (*light blue*), detergent (*orange*), and oil (*dark blue*) across vacuum sealing by sampling month. While non-vacuum sealed samples in a show **a** consistent decrease during the trial, vacuum sealed samples maintain the germination rate of the seeds over the trial. Oil and bleach treatments in **b** both have negative trends reducing germination over the course of the year, while other treatments appear to maintain germination comparable to the original baseline germination (86%). Error bars represent ± standard error of the mean

within sample bags over time may explain why some better prevented bruchid population growth than others. While large detergent particles may not easily bind to seeds, smaller particles, such as the galangal powder, charcoal, and carbaryl, may have evenly coated seeds and caused an additional barrier to counter predation (Van Huis 1991). Additional studies could examine ranges of particle sizes of homemade charcoals, chemicals ground from roots and tubers such as other ginger relatives, and purchased detergents. Most importantly, studies within different regions could focus on local treatments that are readily available and cost-effective to the smallholder farmer in those regions.

3.2 Seed viability

Seed viability and vigor were analyzed by calculating both the seed germination rate and the mean time to 50% germination at each sampling month. Seed with bruchid damage and without bruchid damage were germinated together, and there was no significant difference ($F = 2.37$, $P > 0.05$) between germination rates of damaged and undamaged seed. The main effect of vacuum sealing on seed germination was not significant ($F = 2.02$, $P > 0.05$) across the entire trial, but the trend of germination rate loss of non-vacuum samples led to significant differences ($F = 7.58$, $P < 0.01$) between vacuum-sealed and non-vacuum-sealed treatments by the final sampling month (M12) (Fig. 3a and Table 1).

Although pest damage reduces seed endosperm reserves, perhaps the greater issue facing the smallholder is viability of saved seed. Vacuum-sealed samples maintained viability after 1 year; however, they were not significantly different ($P < 0.05$) from non-vacuum-sealed samples until the final sample month (M12) (Fig. 3a). Significant differences by the vacuum-sealed treatment were not present until the final sample period, but the negative trend of viability alone within non-vacuum-sealed samples suggests that exposure to air, even once a month, increases respiration rates of dormant seeds and leads to reduced viability more rapidly than vacuum-sealed samples (Table 1). Although seed stored within vacuum-sealed bags had little exposure to seasonal conditions, seed removed for viability testing would be influenced by larger weather patterns such as lower humidity, day length, and lower temperatures during the trial. This is especially notable during the third sampling period (M6), in which November saw a drop in humidity, temperature, and rainfall (Fig. 4a, b).

Local seed storage treatments had consistent significant ($F = 200.77$, $P < 0.001$) effects on seed germination rate during all sampling months (Fig. 3b). Oil treatment quickly killed seeds and reduced the germination rate (<20% viability) following application and showed a negative linear trend after the first sampling month (M1). The immediate drop of viability is perhaps caused by oil coating the seeds, preventing respiration and healthy metabolism during storage and germination. Likewise, bleach treatment showed a negative linear reduction of viability throughout the study. Although bleach solutions create toxic alkaline environments when applied to surfaces, the bleach treatment used in the experiment was applied, rinsed away from the seeds, and then seeds were allowed to dry prior to storage. Any residual humidity within vacuum-sealed bags and increase in seed moisture content after rinsing them in bleach and rinse water may negate the positive effects of vacuum or low-temperature storage

Table 1 Trendline regression equations and coefficients of regressions for the effects of locally available seed storage treatments and vacuum sealing treatments across sampling periods on the dependent variables of mean seed germination rate and mean time to 50% seed germination

Treatment	Equation	R^2 value	Best fit
Mean seed germination rate			
Bleach	$y=-6.8128x+89.313$	0.7369	Linear
Botanical	$y=1.5714x^2-9.5286x+101.25$	0.4089	Quad
Carbaryl	$y=1.0408x^2-5.0949x+94.129$	0.6150	Quad
Charcoal	$y=-1.159x^2+7.6042x+78.959$	0.5247	Quad
Control	$y=1.2857x^2-6.8643x+96.2$	0.4592	Quad
Detergent	$y=-2.4909x^2+8.1325x+71.877$	0.8749	Quad
Oil	$y=-3.8x+19.3$	0.7313	Linear
Mean time to 50% seed germination			
Bleach	$y=-0.4805x^2+2.6961x+1.2911$	0.4061	Quad
Botanical	$y=-0.4102x^2+2.2665x+2.2297$	0.4323	Quad
Carbaryl	$y=-0.5895x^2+3.4164x+0.7828$	0.5651	Quad
Charcoal	$y=-0.5621x^2+3.4063x+0.3532$	0.6121	Quad
Control	$y=-0.5459x^2+3.1163x+0.9855$	0.5330	Quad
Detergent	$y=-0.4838x^2+2.7304x+1.5941$	0.5397	Quad
Oil	$y=-0.5755x^2+2.6965x+2.06$	0.9449	Quad
Mean seed germination rate			
Vacuum	$y=0.9847x^2-5.901x+82.057$	0.2669	Quad
No vacuum	$y=-0.6791x^2+0.964x+77.476$	0.8901	Quad
Mean time to 50% seed germination			
Vacuum	$y=-0.5126x^2+2.9103x+1.1989$	0.5582	Quad
No vacuum	$y=-0.5295x^2+2.8978x+1.4572$	0.6835	Quad

environments and lead to loss of viability. All other treatments (botanical, charcoal, carbaryl, and detergent) maintained germination rates that were comparable to the baseline germination rate (86%), offering a promising starting point for smallholder farmers to maintain seed viability through the use of locally available and inexpensive seed treatments often passed down through indigenous knowledge. Overall, vacuum sealing shows much promise for the simultaneous maintenance of seed viability while controlling pests in stored seeds without the use of expensive technologies or harsh chemicals, on which prior work has focused.

3.3 Seed vigor

Total vigor of viable seeds, measured as mean time to 50% germination, followed similar patterns across both the main effect of vacuum sealing and the main effect of local seed storage treatment (Fig. 4 and Table 1). There were no significant differences in vigor between treatments for the main effect of vacuum sealing and inconclusive significance between local seed storage treatments.

During the sixth sampling month in November, seed germination across both the main effects of vacuum and local seed storage treatments was likely delayed due to changing weather patterns, namely, reduced rainfall, decreasing temperatures, and reduced humidity (Nov, M6; Fig. 4). However, in the months following the sixth sampling (M9, M12), there is an overall decrease in the time needed for germination, which is most likely due to warming temperatures, an increase in humidity, returning rainfall, along with increasing day length (Fig. 4).

The effect of changing climatic conditions (from October to February) on seed vigor is most apparent in the main effect of vacuum sealing (Fig. 4c) and the main effect of local treatments (Fig. 4d) by increasing mean time to 50% seed germination. An increase in mean time to 50% germination during month 6 suggests that seeds are reacting negatively to lower temperatures, light, and humidity (Fig. 4a, b). Instead of a loss of seed viability throughout the duration of the study and increasing the days to 50% germination, both non-vacuum- and vacuum-sealed samples appeared to maintain seed vigor by the end of the study, suggesting that the return of wet season humidity, temperatures, and increasing day length had influence on germination trials (Fig. 4).

4 Conclusion

Resource constraints of smallholders and organizations working at the local community level will continue to influence how seeds are stored in the tropics and subtropics. We have shown that small-scale seed banks and farmers that need to

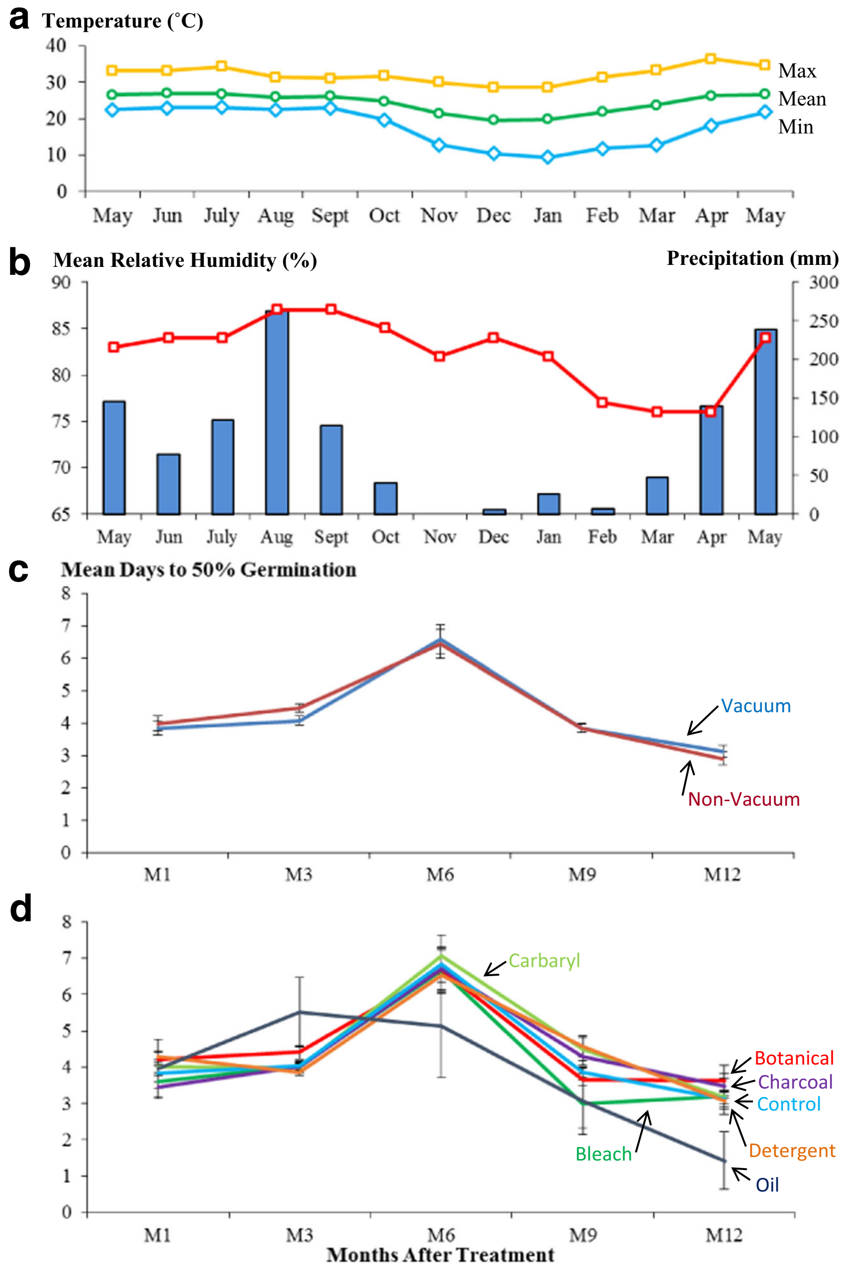

Fig. 4 Mean climatic conditions of **a** maximum (*yellow*), mean (*green*), and minimum (*blue*) temperatures (°C) and **b** mean relative humidity (*red*), and mm precipitation (*blue*) of Chiang Rai, Thailand (19° 54' N 99° 49' E) for the months of May 2011 through May 2012, and mean time to 50% germination for **c** vacuum (*blue*) and non-vacuum (*red*) sealed treatments across locally available seed storage treatments by sample month and **d** locally available seed storage treatment across vacuum sealing treatments for bleach (*dark green*), botanical (*bright red*), carbaryl (*light green*), charcoal (*purple*), control (*light blue*), detergent (*orange*), and oil (*dark blue*) by sample month. Error bars represent ± standard error of the mean. The increase of mean time to 50% germination during the sixth sample month (M6-November) is attributed to lack of rainfall, reduced humidity, and reduced temperatures during the beginning of the dry season

control granivorous insects similar to bruchids in stored seed could benefit by utilizing vacuum storage that prevents insect population growth without the use of expensive, low-temperature, and low-moisture seed storage conditions, on which most prior work has focused. In addition, we have shown that vacuum sealing continues to prove its utility by maintaining seed viability in tropical locations, while local seed treatments, (excluding bleach and oil) appear to not interfere with seed viability when included in storage. This work is novel in that it combines both locally available treatments with low-cost vacuum sealing to not only control cowpea bruchids but also prolong viability of stored seeds in a resource-constrained tropical setting—analogous to the conditions faced by the world's 500 million smallholder farmers and the many NGOs working with them for improving food security. We have demonstrated that vacuum sealing and several of the local treatments simultaneously provide novel, low-cost, sustainable, and appropriate seed storage and insect pest control options for smallholder farmers and seed banks in the developing world.

Increasing the self-reliance and seed quality of a smallholder or small community seed banks through low-cost tools such as vacuum sealing or the utilization of local seed storage treatments could have dramatic influence on increasing food security, maintaining biodiversity of global food crops and seed varieties, and increasing the available exchange of regionally specific crops. As small-scale farmers produce the majority of food for populations within developing countries, additional research focusing on these appropriate methods of storage for the farmer could help increase food security and preserve crop biodiversity.

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