Efficacy of botanicals and *Bacillus thuringiensis* to control potato tuber moth, *Phthorimaea operculella* (Zeller), in potato stores in Nepal

Giri, Y.P.[1], **R. Maharjan**[1], T. Dochen[2], K. Nidup[2], M. Sporleder[3], and J. Kroschel[3]

[1]Entomology Division (NARC), [2]Bhutan Potato Development Program (BPDP), [3]Centro Internacional de la Papa, Integrated Crop Management Division, Apartado 1558, Lima 12, Peru

Abstract

In Nepal potato is an important food security and a primary cash crop for many small-scale farmers. Potato production has steadily increased since the last decades; however, pests are major constraints limiting the yield potential and causing potato yield losses of up to 80%. Among limiting biotic factors, the potato tuber moth (*Phthorimaea operculella*) has become the major pests in potato cropping in Nepal. Current farmers' control practices rely on the use of highly toxic pesticides applied with little or no protective measures, causing substantial adverse impacts on human health and the environment. The objective of our study was to test the efficacy of two botanicals, crushed leaves and stems of *Artemisia* sp. and an extract of *Acorus calamus* rhizomes, in comparison to a commercial product of *Bacillus thuringiensis* var. *kurstaki* (*Btk*) in rural potato stores in the mid-hills of Nepal and Bhutan. Potato tuber moth damage to tubers was evaluated in monthly intervals for a period of 4-5 month. Proportions of damaged tubers were highly related to the density of mines per tuber. Further, the distribution of damage in potato heaps was slightly aggregated. Our results showed that all treatments significantly reduced tuber damage. The rhizome powder of *Acorus calamus* provided best protection followed by *Btk* and *Artemisia*. Damage in potato heaps treated with *Artemisia* increased gradually while *Acorus calamus* and *Btk* delayed infection increase for about 1-2 month. None of the products provided satisfying protection after 3-4 month storage period at severe potato tuber moth pressure but might be efficient, when storage condition do not allow a reinfestation by moths.

Keywords: Farmers' Storage, botanicals, formulations, eco-regions, potato tuber moth, Nepal.

Introduction

Potato (*Solanum tuberosum* L., Solanaceae) is an important food and cash crop of Nepal. The crop is grown in various cultivation systems year-round and from high hills to plain areas throughout the country. Threat of insect pests is increasing with intensification of cultivation. The potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera; Gelechiidae), is one of the major pests attacking potato. The pest, which is native to South Americas, is today cosmopolitan in distribution. In Nepal, it was reported from Kathmandu (Shankhu), Kavrepalanchowk (Banepa, Nala, Panchkahal), Nuwakot (Trisuli), Dhading (some places), Makawanpur (Palung) and Bara (Parawanipur) districts (Joshi, 1989, 1994). The larvae mine into potato leaves and tubers. Most important is the tuber damage in potato storage that leads to tuber weight losses and rotting (due to infection of fungi or bacteria). In storage, up to 90% weight loss is possible (Joshi, 1989) while infestation of tubers might reach 100% (Lal, 1987, 1998; CIP, 1988; Palacios and Cisneros, 1996). At present, many Nepalese farmers use broad-spectrum chemical pesticides, including organochlorines, to manage potato tuber moth in the field and storage, often with unsatisfying results. Considering the negative health and environmental impacts arising from the unwise use of chemical pesticides in such situations, it is crucial to develop alternative control measures for managing potato tuber moth.

Botanical insecticides have long been used in traditional pest management before the invention of chemical pesticides. They reputedly pose little threat to the environment and to human health and could still play an important role in modern pest management system. In Nepal, 324 plant species are recognized having insecticidal properties (Neupane, 2000). Still many farmers continue to control potato tuber moth by using indigenous control practices like the use of plant material. Kennedy (1984), Pradhan (1988), and Rivera and Retamazo (2000) reported some plants and weeds like Muna (*Minthostachys* sp.), Eucalyptus (*Eucalyptus globulus*), Chilca (*Baccharis* sp.), curry plants, Indian pivets, *Lantana camara*, Pangam leaves, *Chenopodium botrys*, *Mentha arvensis* and *Artemisia vulgaris* (Asteraceae), *Lycopersicon hirsutum* among others, are effective in controlling potato tuber moth. Currently, many farmers cover their potato with *Artemisia* spp. for protecting them from potato tuber moth attack. Kroschel and Koch (1996) reported also of a significant reduction of tuber infestation by covering potatoes with leaves of the trees *Schinus molle* and *Eucalyptus* sp.

In the present study, two plants, sweet flag (*Acorus calamus)* and *Artemisia* sp. have been evaluated against potato tuber moth in rustic potato storerooms. *Artemisia* spp. is an herbal plant naturally distributed in mid hills of Nepal in wood edges, prairie restorations, herbal gardens, and miscellaneous waste places. It usually occurs in disturbed areas, but has displayed a capacity to invade more natural sites. For protecting the potato tubers, fresh or dried plant parts are used which act as repellent. Sweet flag (Acoraceae), native to India, central Asia, and Eastern Europe is found today in many temperate and sub-temperate areas of the globe. In Nepal, the herb is available up to 2000 meter altitude. Habitats include sedge meadows that are prone to flooding, edges of small lakes and ponds, marshes, swamps, seeps and springs, and wetland restorations. The plant contains β-asarone in stolons which is considered the main substance that acts as insecticide. Content of β-asarone in the plant varies according maturity and altitude where it has grown (Paneru et al. 1997). The two plants were tested along with a bio-pesticide based on *Bacillus thuringiesis* var. *kustaki* (*Btk*) formulated in talcum for comparison. *Btk* formulated in talcum powder or in quartz-rich fine sand and dusted over stored potato has been reported to provide good protection against the potato tuber moth (Lacey and Kroschel 2009, Kroschel and Koch 1996, Raman et al. 1987).

Materials and methods

Experimental sites

The experiments were conducted in 11 farmer's rustic potato storerooms in five different commercial potato growing areas of the central mid-hills of Nepal and Bhutan (Table 1).

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Table 5. Experimental locations in Nepal and Bhutan

Country	District	Sites	Altitude (msl)	No. of experiment	Used in analysis
Nepal	Sindhupalchowk	Thumpakar	2513	2	1, 2
	Makwanpur	Palung	1798	3	Excluded*
	Kavrepalanchowk	Banepa	1454	1	Excluded*
		Dhulikhel	1600	1	Excluded*
		Nala	1588	4	3, 9, 10, 11
Bhutan	Trashigang	Yangneer	1861	1	13
	Mongar	Drametse	1867	1	Excluded*

*Storages were excluded from the analysis because damage pressure by the potato tuber moth was low

In addition, *Acorus calamus* along with the *Btk*-talcum product was tested in two farmers' storerooms in Eastern Bhutan. *Artemisia* was not tested in these sites because the plant was not available during the time of experimentation.

Description of treatments and experimental processes

Artemisia: Mature leaves with stem were collected, shadow dried for 6 days and cut into pieces of 1 cm size in order to make it ready for application. The material was mixed with non-infected potato tubers at the rate of 20 g/kg potatoes.

Sweet flag (*Acorus calamus*): stolen were collected; shadow dried and crushed to a fine dust by using an electrical crusher. The material was spread over the stored potatoes at the rate of 5 g/kg potatoes.

Btk: Commercially available *Btk* (Z-52-strain, Biolep, India) was first mix at a rate of 35 g per liter water. Talcum powder was added to the solution at a 1:1 (w/w) ratio and mixed thoroughly. The paste was poured on the plastic sheet and air dried in the shadow. After drying, the mixed material was crushed into fine powder using a kitchen role. The final product was applied at a rate of 6 g per kg potato tubers. Batches of 15 kg tubers were shaken together with the *Btk* powder in plastic bags until the tubers were uniformly covered with the dust formulation.

A total amount of 60 kg potatoes were used for each treatment and the control (without any protection). Potatoes for each treatment were piled in one corner of the farmers' storeroom exposed to naturally occurring potato tuber moth attack. Damage was evaluated over a period of about 5 month (from 2nd July to 19th November, 2008). In an interval of about one month 100 tubers were removed from each treatment and brought to the laboratory for assessing total numbers of infested tubers (without typical symptoms of potato tuber moth attack) and number of mines per tubers (sampling was destructive). For logistic reasons the evaluation interval was not always exactly one month.

Statistical analysis

The model of Nachman (1981, 1984) was fitted to the data from each sampled unit for determine the relationship between mean infestation (i.e. the mean number of mines per tuber) and the proportion of tubers damaged. The model is:

$$P_a = 1 - \exp\left(-a\ m^b\right)$$

in which P_a in the proportion of damaged tubers and *m* is the mean number of mines per tuber in the sample unit. For determining the relationship between the variance, s^2, and the mean, *m*, of mines per tuber Taylors' power law (1961) ($s^2 = a\ m^b$) was used. The parameters *a* and *b* in the equation were estimated by linear regression after logarithmic linearization of the equation.

Data on damage levels (proportion of damaged tubers per sampling unit) were submitted to 3-way ANOVA; including the factors 'treatment', 'storage' and 'evaluation month' in the model. Since the data include one value only for each factor combination main effects only were evaluated (interaction between factors was not evaluated). Post analysis differences between groups (main effects only) were analyzed using Tukey-test ($p < 0.05$). In some storages damage levels of potato tuber moth were very low and populations did not develop during the course of experiments; these storages were excluded before analysis (Table 1). For multiple comparison chi-square test was applied to all pairs of samples (treatment × storage × sampling date) testing if the proportions of damaged tuber were equal. The test statistic was:

$$\chi^2 = \sum \frac{(O_i - n_i p)^2}{n_i pq}, \text{ for } i = 1, 2$$

where O_i was the observed frequency of damaged tubers for the i^{th} sample, n_i was the samples size for the i^{th} sample, p was $\Sigma O_i / \Sigma n_i$, and q was $1 - p$. *Chi*-square values were evaluated using 1 *df*, and $p = 0.05$.

Results and discussion

Mean infestation versus proportion of damaged tubers

The Nachman model described well the relationship between the frequency of damaged tubers and the damage density (mean number of mines per tuber) (Figure 1a).

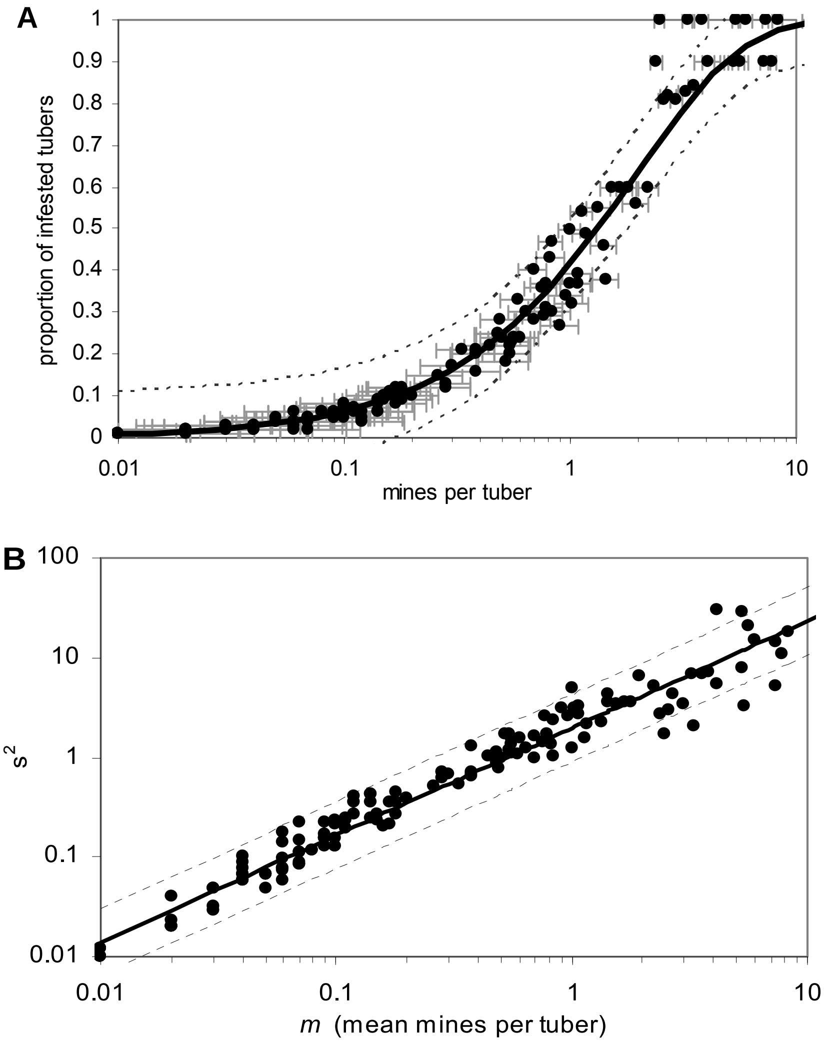

Figure 1.(A) Relationship between proportion of damaged tubers (P_a) and mean number of mines per tuber (*m*). Points are observed data, bars indicate the standard variation of the mean (*m*), bolt line is the Nachman function fitted to the data, and scattered line indicates 95% confidence bands. (B) Exponential response of the variance (s^2) to the mean number of mines per tuber (*m*). The function has the form log(s^2) = 1.074 log(*m*) + 0.2931, R2 = 0.9615.

The values of the parameters *a* and *b* (equation 1) were 0.578 and 1.001, respectively (R^2 = 0.9673). Similar results were obtained by Roux *et al.* (1992) who fitted the same model to data collected in Tunisian potato storerooms infested by potato tuber moth (he obtained parameter values of *a* = 0.687, and *b* = 0.932 with an regression coefficient of R^2 = 0.994). This substantiates the strong relationship between the infestation density and proportion of damaged tubers and that the Nachman model is highly suitable for describing this relationship. It implies that one of the two variables alone, frequency of damaged tubers or mines per tuber, can be use to describe potato tuber moth damage levels in potato storerooms.

Application of Taylors power law ($s^2 = a\ m^b$) showed that variance of the number of mines per tuber highly depends on the mean infestation (Figure 1b). The coefficients obtained (*a* = 1.964 and *b* = 1.074, R^2 = 0.9615) were quite similar to the coefficients obtained by Roux *et al.* (1992) (*a* = 1.786 and *b* = 1.1, R^2 = 0.99) that indicates a slight aggregation of the damage. Roux *et al.* (1992) also showed that the distribution of damage does not follow the Poisson distribution.

Efficiency of treatments

The potato tuber moth infestation density was very variable among the potato storerooms. Results from some storerooms were not included in the analysis because potato tuber moth was absent or did not develop substantial populations in these storages. Finally, results from 7 storerooms (6 in Nepal, 1 in Bhutan) were evaluated (Table 1). ANOVA, applied to the proportions of damaged tubers (number of mines were not further analyzed because the exact number could not be assessed well from heavily infested rotting tubers) revealed significant differences among the storerooms ($F = 8.4$, $df = 6, 97$, $p < 0.001$), while infestation levels significantly changed (increased) during the course of experiments ($F = 18.1$, $df = 4, 96$, $p < 0.001$). The effects of treatments were significant ($F = 19.8$, $df = 3, 96$, $p < 0.001$). The separation by using the Tukey-test ($p < 0.05$) showed that all three treatments, *Acorus calamus*, *Artemisia* and *Btk* significantly reduced damage levels compared to the control. *Acorus calamus* provided best protection and reduced overall mean infestation to 24%, while overall infestation in *Artemisia* and *Btk* treated potato piles was 39.5% and 36.6%, respectively, compared to an overall infestation of 69.7% in controls. However, since the variation in proportions of damaged tuber is not expected to follow the Poisson distribution and due to the fact that the increase of damage did not follow the same trend in all plots (interaction between the main factors could not be analyzed), the results from the ANOVA might be misleading. Therefore all pairs of samples were additionally compared by using the chi^2-test and separated into equal groups based on this analysis (**Table 6**). The results show that *Acorus calamus* in all storages provided the best protection and in some cases significantly higher then *Artemisia* and *Btk*. Overall damage levels in tubers increased with time of storage. In controls proportions of infected tubers increased gradually from almost zero at the beginning of the experiment to 100% after about 4 month in most storerooms. In *Artemisia* treated piles increased damage was already visible at the beginning of the experiment but at a lower rate than in the controls. In contrast *Acorus calamus* and *Btk* delayed the increase of infection for about 1-2 month; after that damage level increases as well in these treatments. Therefore, none of the treatments provided full protection of tubers, but might be used with satisfying results when potatoes are stored for short periods (2-3 month) only. In these experiments, the potato tuber moth density increase in each storeroom was most probably due to the development of potato tuber moth in the control tubers, and therefore the protective capacity of the treatments, especially of *Acorus calamus* and *Btk*, might be more satisfying even after a longer period of storage if all stored tubers are treated with the product. Kroschel and Koch (1996) showed that *Btk* mixed with fine sand was efficient for a storage period of more than 3 months. In areas where the potato tuber moth occurs, good storage practice is necessary to prevent an influx of moths and hence infestation of young unprotected potato sprouts. Without these additional methods, managing the potato tuber moth effectively with *Btk* or other biological methods in potato stores over a longer period time (four to five months) will be impossible (Kroschel and Sporleder 2006).

Niroula and Vaidya (2004) tested powder of sweet flag rhizomes (*Acorus calamus*) at three concentrations (0.05%, 0.5% and 5% w/w) against potato tuber moth and found 56.7%, 66.7% and 70% adult mortality after 168 hours of treatment application, respectively. Pradhan (1988) tested indigenous weeds against potato tuber moth under farmer's storage condition and recommended chopped and dried leaves of *Eucalyptus* sp. (Masala), *Artemisia vulgaris* (Titepati) or *Chenopodium botrys* at a rate of 300-330 g/crate with 8 kg of potato. Further, a dust preparation of *Acorus calamus* rhizomes applied at 10 g/kg potato was efficient to manage potato tuber moth similar to applications of the botanical Marauta (*Chenopodium* sp.). Tiwari (2006) reported of the high efficacy of *Btk* for controlling potato tuber moth.

Table 6. Proportion of damaged tubers in seven farmer's rustic potato storerooms after treatment with *Acorus calamus*, *Artemisia* sp and a *Btk*-powder compared with a control

Storage	Time (days)	Control		*Acorus calamus*		*Artemisia*		*Btk*	
1	0	0	a						
	32	86	h	8	bc	36	e	47	ef
	76	71	g	24	de	72	g	28	de
	101	90	h	56	f	77	gh	49	ef
2	0	0	a						
	13	0	a	1	ab	1	ab	1	ab
	44	35	de	6	bc	4	b	10	bc
	76	100	-	14	cd	8	bc	10	bc
	110	100	-	2	ab	50	f	100	
	140	100	-	90	h	90	h	90	h
3	0	1	ab						
	13	38	ef	1	ab	10	bc	2	ab
	44	100	-	3	ab	12	c	5	bc
	76	100	-	0	a	0	a	0	a
	110	100	-	100		100		100	
9	0	33	de						
	7	23	d	2	ab	60	fg	20	cd
	44	90	h	29	de	84	h	82	gh
	76	90	h	20	cd	90	h	90	h
	110	100	-	81	gh	100		100	
	140	100	-	81	gh	0		100	
10	0	6	bc						
	7	24	de	2	ab	21	cd	13	cd
	44	55	f	2	ab	5	bc	15	cd
	76	28	de	2	ab	2	ab	7	bc
	110	100	-	30	de	30	de	25	de
	140	37	ef	24	de	22	cd	27	de
11	0	6	bc						
	7	22	cd	3	ab	37	ef	9	bc
	44	100	-	10	bc	43	ef	11	bc
	76	100	-	60	fg	4	b	10	bc
	110	100	-	18	cd	38	ef	39	ef
	140	100	-	6	bc	32	de	34	de
13	60	12	c						
	91	18	cd	8	bcd	0	a	2	ab

Figures with identical letters are not significant different according to the pair wise evaluation using the Chi^2 test ($P < 0.05$).

Conclusions and recommendations

Acorus calamus stolon dust at 5 g/kg of potato tubers showed high efficacy to protect potato tubers against potato tuber moth for about three to four month in farmer's rustic potato stores. Further studies should address the preparation technique and dosage for using of *A. calamus.* However, its protective capacity is comparable with an already recommended bio-pesticide based on *Btk* in a powder formulation. *Btk* is commercially available but need to be mixed with talcum before application. *Artemisia* leaves chopped pieces (20 g /kg potato) were also effective in reducing potato tuber moth damage levels and therefore this traditional control technique also warrants its application. Therefore, several alternative control methods are available for protecting stored potato from potato tuber moth damage which could be used by farmers in Nepal. Training and regular technical support through the governmental extension services is important to introduce these technologies along with an integrated pest management program to tackle the high risks of pesticide poisoning in resource-poor farm households in Nepal.

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