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Agriculture Research and Innovation Division
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Thimphu : Bhutan

Editorial

The Bhutanese Journal of Agriculture welcomes the submission of research articles and short communications. The article may be submitted to *bja@moaf.gov.bt*. Please follow the journal format provided in the author guidelines, accessible from *www.bja.gov.bt* or *www.doa.gov.bt*. The online open access for the Bhutanese Journal of Agriculture is now available at *www.bja.gov.bt*.

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FOREWORD

The Department of Agriculture is delighted to launch the sixth volume of the Bhutanese Journal of Agriculture (BJA). BJA is a print open-access English language journal on agriculture and publishes research articles annually with the primary purpose of providing an additional mechanism to disseminate appropriate technologies, and knowledge and information in the agriculture sector.

Our editorial team, comprising members entirely from within the Department of Agriculture, is pushing hard to conform to international standards. Concerted efforts are underway to continuously improve the quality of the journal and we are glad that with every passing issue, we have come out a step better. The experience has undoubtedly enriched our colleagues who seem to be closing the gap in designing research, carrying out analysis and putting across effective communication of their results, which in the final analysis underpin agriculture development.

Following our successful DOI (Digital Object identifier) registration as a member with an authorized DOI provider in 2022, the journal papers in this volume are provided with DOIs. This will enable easy indexing and accessibility of our papers online while ensuring their long-term storage in the digital space as well as enhance our journal's credibility.

I thank the authors and the reviewers for their contribution as well as the BJA Editorial Board for their added efforts in successfully taking out this edition. I wish everyone a resourceful reading.

Tashi Delek and best wishes!

(Yonten Gyamtsho)
DIRECTOR

EDITORIAL

The Bhutanese Journal of Agriculture (BJA) focuses on original research results that help generate technologies targeted at improving agricultural processes, increasing crop yields and conserving agricultural resources in the Bhutanese context. The COVID-19 pandemic has underscored and reiterated the need to build a resilient and locally sustainable food production and distribution system to reduce dependency on imports. The strengths and positive attributes of domestic food production have become clearer as substantial quantities of vegetables that we consume now originate domestically. Hence, we hope that the articles presented in this edition will add to the current understanding of, and thereby help enhance food security and food systems without depleting our resource base.

The journal received 19 manuscripts that were reviewed by 17 experts including the editorial board members. The review reports were deliberated by a panel of reviewers in a three-day workshop. Through a rigorous revision process including strict compliance with the journal guideline, only 3 manuscripts have been accepted and are featured in this volume.

Once again, we thank all authors, reviewers, facilitators and the journal editorial board for their concerted effort and diligence in making this volume happen. On behalf of the editorial board, I would like to extend our sincere gratitude to all contributing institutions including the Agriculture Research and Development Centres at Wengkhar, Samtenling and Bajo; the National Post Harvest Centre, Paro; the Agriculture Machinery and Technology Centre, Paro; and the National Centre for Organic Agriculture, Yusipang. I put on record my appreciation to the Agriculture Research and Innovation Division and the DoA for providing the resources required to not only implement the research but also to make this edition a success. We hope that such an earnest effort to document evidence-based studies and their results will lend added credibility to our plans and programs aimed at helping us realize our mission to secure food for all Bhutanese people.

We wish you an intuitive reading.

(Wangda Dukpa)
Interim Editor-in-Chief

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Efficacy of Plant Derivatives in Protecting Mungbean Grains against Cowpea Weevil (*Callosobruchus maculatus*) under Storage Conditions in Southern Bhutan

Chinta Mani Dhimal[1], Ratu Kinley[1]

ABSTRACT

Food grains infestation by insect pests in stores is a severe challenge in food production around the globe, especially in wet and humid regions. In Bhutan, mung bean is commonly grown for consumption as a superior source of protein. However, severe cowpea weevil infestation is observed while in storage condition. Controlling with synthetic pesticides is associated with health risks due to toxic residues which intervene in safe and healthy protection methods. Protecting the grains in-store through organic approach is imperative for consumption and seed purposes. Some botanical plant extracts are known for their protective properties which need location-specific studies based on availability and suitability. Therefore, this study was conducted to evaluate the efficacy of eight treatments (Acorus calamus rhizome powder, mustard oil, garlic cloves, turmeric rhizome powder, wood ash, Vitex negundo leaf powder, super grain bag including untreated control) against cowpea weevil (Callosobruchus maculatus) in mungbean under storage condition. The experiment was laid in a randomized complete block design with three replications. The result revealed that the lowest mean number of grains perforated and percent grain perforated were recorded in grains treated with Acorus calamus rhizome powder (0.46 & 0.20 %) followed by mustard oil (1.05 & 0.47 %) and grains stored in super grain bag (5.74 & 2.49%), which were significantly lower (P<0.001) as compared to other treatments. The lowest number of adult cowpea weevils was also recorded in grains treated with Acorus calamus and mustard oil followed by grains stored in super grain bag. Germination percentage was found highest in wood ash followed by mustard oil, Acorus calamus and garlic-treated grains while the lowest was in super grain bag. Therefore, Acorus calamus rhizome powder and mustard oil were found to be effective in managing cowpea weevil without affecting seed germination and vigour.

Keywords: *Cowpea weevil; Mung bean; Management; Treatment*

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1 Introduction

Insect infestation and damage to stored grains are severe challenges in food production around the globe. The stored food grain damage ranges from 5% to 30% of the total production globally (Pugazhvendan, Elumalai, Ronald Ross, and Soundararajan, 2009). The reasons for the widespread of insects are due to their evolutionary adaptation in terms of morphological and physiological behaviours created by human actions providing suitable habitat within the food stores. These insects are mostly found in storage, processing, packaging and other post-harvest processes. The storage insects commonly cause substantial damage to the stored grains due to their ability of high reproductive potential especially in warm areas due to the conducive environment (Ahmad et al., 2021).

Many insects such as cowpea weevil (*Callosobruchus maculatus*), lesser grain borer (*Rhyzopertha dominica*), granary weevil (*Sitophilus granarius*), rice weevil (*Sitophilus oryzae*), angoumois grain moth (*Sitotroga cerealella*), rust-red flour beetle (*Tribolium castaneum*), confused flour beetle (*Tribolium confusum*), saw-toothed grain beetle (*Oryzaephilus surinamensis*), flat grain beetle (*Cryptolestes spp.*), warehouse moth (*Ephestia spp.*), Indian meal moth (*Plodia interpunctella*), warehouse beetle (*Trogoderma variable*), broad horned flour beetle (*Gnatocerus cornutus*), cadelle beetle (*Tenebroides mauritanicus*) coffee bean weevil (*Araecerus fasciculatus*) and others are responsible for the infestation and damage of food grains in storage (Banga, Kumar, Kotwaliwale, & Mohapatra, 2020; Deshwal et al., 2020). Among these, the Cowpea weevil (*Callosobruchus maculatus*) is one of the major pests affecting economical legume crops such as cowpea, lentils, green gram and black gram in storage condition (Devi & Devi, 2014).

Cowpea weevil (*Callosobruchus maculatus,* Fabricius, 1775) is taxonomically classified under Domain: Eukaryota, Kingdom: Metazoa, Phylum: Arthropoda, Subphylum: Uniramia, Class: Insecta, Order: Coleoptera, Family: Bruchidae, Genus: *Callosobruchus* and Species: *maculatus*. This insect is globally called by different names such as Cowpea seed beetle, Four-spotted bean weevil, Southern cowpea weevil or Spotted cowpea bruchid. The adult weevils are about 2.0 to 3.5 mm long having slightly serrated antennae in both sexes. Female adults have strong markings on the elytra with two large lateral dark patches at mid-way along the elytra and smaller patches at the anterior and posterior end leaving a pale brown area resembling a cross. Males are less distinctly marked on their elytra as compared to females. The doomed-shaped egg has an oval and flat base which attaches to the surface of the pulses grain and measures about 0.47mm long and 0.12mm wide. Fully grown larvae size is about 3.64 mm long and 2.00 mm wide and the size of the male and female pupa is about 4.07 mm long, 2.23 mm wide and 4.57 mm long and 2.60 mm wide respectively (Devi & Devi, 2014).

During the early stage of damage, the visible symptoms are not exposed except for the presence of eggs, as they are attached to the surface of grain enclosed by an egg case. The newly hatched larvae start feeding on the grain and perforate inside the grain. Upon completion of its life cycle, the adult emerges through a circular hole, the only observable symptom on the grain. The weevil can breed throughout the year and takes about 45 to 48 days to complete one life cycle under favourable conditions depending on the availability of food materials (Devi & Devi, 2014). The egg stage duration ranges from 6 – 7 days, the larvae stage ranges from 18 to 22 days, and the pupa ranges from 5 to 7 days (Devi & Devi, 2014). The adults do not feed on the stored grains and have a life span of about 12 days. During this short period, the female lays about 115 eggs on the surface of the grains with a firm glue-like substance (Devi &Devi, 2014). As per the study conducted by Moreno, Duque, De la Cruz, and Tróchez (2000), the average female oviposition period is about 10.2 days. The temperature range between 18.14^{o}C to 27.14^{o}C and humidity of 79.5% is suitable for oviposition (Devi &Devi, 2014).

There is much research conducted on the use of different insecticides for the management of cowpea weevil in stored grains (Braga et al., 2007; Visarathanonth, Khumlekasing, & Sukprakarn, 1990). Continuous and indiscriminate use of pesticides has not only led to the development of resistant strains but also the accumulation of toxic residues in food grains used for human consumption (Rajapakse, 2006; Said & Pashte, 2015). Globally, there are serious problems of pest resurgence, genetic resistance of insects, residual toxicity in crops, phototoxicity, vertebrate toxicity, environmental hazard, and increased cost of pesticides due to which there is a need for effective alternatives to synthetic pesticides (Rahman & Talukder, 2006; Uzair et al., 2018). Such issues have diverted pest control approaches from conventional towards the use of plant derivatives, which are eco-friendly and safer alternatives for seed storage and consumption.

Botanical extracts of many plants have antifeedant, repellant and ovicidal properties on insects and affect insect growth and development due to which they can be used as safer and eco-friendly alternatives for the management of storage insect pests (Haridasan, Gokuldas, & Ajaykumar, 2017; Rajapakse, 2006; Said & Pashte, 2015). Moreover, they are readily available to farmers and they can be prepared locally. Many botanical plants such as *Vitex sp.*, turmeric

(*Curcuma longa*), *Brassica compestris*, garlic (*Allium sativum*) & neem (*Azadiratchta indica*) have been found to be effective in the management of storage pests (Said &Pashte, 2015).

As per the RNR Census of Bhutan, (RSD, 2019), 14.33 percent of households are facing problems with crops damaged by pests and diseases which is inclusive of storage pests. Like in other countries, storage pest causes serious post-harvest losses for smallholder farmers of Bhutan, who use traditional storage methods and structures for grain storage. Storage pests not only damage the food grains but also reduce the quality of stored products with the presence of insects and their feedings in the products. There are about 49 storage insect pests recorded in Bhutan out of which *Sitotroga cerealella*, *Sitophilus zeamais* and *Sitophilus oryzae* were found to cause significant damage in major staple cereals like rice and maize (Devi & Devi, 2014). Similarly, the Cowpea weevil also causes significant damage in legume crops like mung beans in storage but no research has been done to quantify and validate the storage loss caused in legumes.

However, an assessment of storage losses in Maize by Dorji, Tshering, and Lhamo (2020) shows that insect infestation is responsible for storage losses up to 16.18% to 38.21% and causes the maximum storage losses in Bhutan. Mung bean is usually stored traditionally in polypropylene bag and jute sags after sun drying in Bhutan. Although modern storage techniques like Super Grain Bags (SGB) have been introduced in Bhutan by the National Plant Protection Centre (NPPC), their adoption is still almost negligible due to limited access to the product in rural farming communities. SGB is an important eco-friendly measure to protect grains in stores by reducing water and oxygen (from 21% to 5%) flow between stored grains and the outside atmosphere.

Post-harvest loss of crops can be minimized by managing the storage insect pests using locally available botanical plant extracts besides adopting good management practices like proper drying to moisture content at 9 to 10% (Mbeyagala et al., 2017), maintaining clean storage facilities, improving storage facilities and use of modern storage technologies like super grain bags. Managing storage insect pests can enhance food security of our marginal farmers by preventing post-harvest loss of grains in storage conditions. Much research has been conducted in other countries for controlling storage pests of grains with many recommendations. There are limited studies and experiments on storage pest management conducted in Bhutan. Therefore, this study aims to evaluate the efficacy of different botanical plant extracts, traditional storage methods and modern storage technology in managing the cowpea weevil (*Callosobruchus maculatus*) in mung bean grains in storage conditions.

2 Materials and Method

2.1 Experimental design and materials

The study was conducted at the Agriculture Research and Development Center (ARDC) Samtenling (26º 54’ 17” N, 90º 25’ 51” E) located at 372 meters above sea level, from February to October, 2020. The experiment was carried out inside room conditions without any controlled environmental factors but with windows open for air circulation. The experiment was conducted using Randomized Complete Block Design (RCBD) with three replications and eight treatments. For each experimental unit, 3 kg mung bean grains were packed in polypropylene bags after the application of each treatment and stored for 195 days (6.5 months). Grains were thoroughly washed, and sun-dried up to 9% moisture content and all the damaged grains were discarded before the application of treatments and storage as per the safe grain storage guidelines by Sharon, Abirami, Alagusundaram, and Sujeetha (2015).

2.2 Preparation of treatments

Eight different treatments were used to evaluate its efficacy against Cowpea Weevil infestation as shown in Table 1. Locally available plant parts were collected from the nearby localities. Local mung bean (*Vigna radiata*) grains were harvested from the research field and used for the study. Polypropylene bags were used for storing the grains as it is commonly used by the farmers in Bhutan for grain storage. For treatments, sweet flag rhizomes (*Acorus calamus*), garlic cloves (*Allum sativum*), wood ash and Chinese chaste tree leaves (*Vitex negundo*) were collected from the locality in Sarpang district. Turmeric rhizome powder and mustard oil were purchased from local shops and super bags (GrainPro®, MSD-DR001-2) were used as per the technical recommendation.

Table 1. Treatment preparation and application

S.N.	Treatment	Application rate	Preparation and application of treatments	Reference
1	Sweet flag (*Acorus calamus*) rhizome powder	50g/Kg	Rhizomes were washed, cut into pieces, shed-dried and ground into powder and mixed with mung bean grains	(Khanal et al., 2021)
2	Mustard oil (Tulsi®)	16ml/Kg	Commercial Tulsi® brand mustard oil was mixed with mung bean grains	(Khanal, Alisha. Khadka, & Rameshwor. Pudasaini, 2020; Mbeyagala et al., 2017)
3	Garlic cloves (*Allium sativum*)	50g/Kg	Individual cloves were separated, sheath were discarded and crushed and mixed with mung bean	(Khanal, *et al*., 2021)
4	Turmeric (*Curcuma longa*) powder (BMC Haldi®)	33g/Kg	Commercial turmeric powder (BMC Haldi®) was mixed with mung bean grains	(Said & Pashte, 2015)
5	Wood ash	33g/Kg	Wood ash prepared from locally available wood (*Schima wallichai* and *Gmelina arborea*) was mixed	(Apuuli & Villet, 1996)
6	Chinese chaste tree (*Vitex negundo*) leaf powder	33g/Kg	Leaves were washed, sun-dried and crushed into powder and mixed with mung bean grains	(Khalequzzaman & Goni, 2009)
7	Super grain bag (GrainPro®)	NA	Standard super grain bag developed by IRRI (GrainPro®, MSD-DR001-2) was used as per technical recommendation	(Tivana et al., 2020)
8	Un treated Control	NA	Grains were stored in the same polypropylene bags without any treatments	NA

2.3 Seed germination test

After 195 days, 100 non-infested seeds from each treatment were selected and tested for seed germination following the paper towel method as per the International Seed Testing Association (ISTA) standard (FAO, 2018). Seed vigour was tested by sowing the non-infested seeds in plastic plug tray using a mixture of soil, compost and sand (ratio2:1:1) as growing media. The seed germination percent and seedling vigour index were calculated as

Equation 1

$$Germination\ Percent\ (\%) = \frac{Number\ of\ seeds\ germinated}{Number\ of\ seeds\ sown} * 100$$

Equation 2

$$Seed\ Vigour\ Index = \frac{Germination\ percentage * Mean\ of\ Seedling\ length\ (cm)}{100}$$

Where; the length of seedlings was measured on the 15th day after sowing.

2.4 Data collection and analysis

In this experiment, the data collection on adult cowpea weevil and its infestation on grains was conducted at regular intervals of 15 days starting from the date of treatment application as conducted by Uddin Ii and Sanusi (2013). On each observation date, 10 grams of grains from each experimental unit were weighed using a high-precision electronic digital weighing balance (WENSAR®) and observed for grain infestation and the number of adult cowpea weevils. The percent grain perforated and Insect Perforation Index (IPI) was calculated as per the methods of Fatope*et al*., (1995) as mentioned by Krishnappa, Lakshmanan, Elumalai, and Jayakumar (2011) (2011) and Ojiako and Adesiyun (2013) (Equation 3 & 4).

Equation 3

$$Percent\ Grain\ Perforated\ (\%) = \frac{Total\ No.of\ grains\ perforated}{Total\ No.of\ treated\ grains} X100$$

Equation 4

$$IPI = \frac{\%\ of\ treated\ grains\ perforated}{\%\ of\ control\ grains\ perforated + \%\ of\ trated\ grains\ perforated} X100$$

If the insect perforation index (IPI) value is above 50, it is an indication of negative protectant ability. Seed germination percent and Seed Vigor Index was calculated as per the method of Shahrajabian, Khoshkharam, Sun, and Cheng,(2019) as per equation 5 & 6, respectively;

Equation 5

$$Germination\ \% = \frac{Number\ of\ germinated\ seeds}{Total\ numberof\ seeds\ sown} X100$$

Equation 6

$$Seed\ Vigour\ Index\ = \frac{Germination\ percentage\ X\ Mean\ of\ seedling\ length}{100}$$

The data was first entered and processed in Microsoft Excel 2007 spreadsheet. Further, it was analyzed using Statistical Tools for Agriculture Research (STAR) version 2.0.1. Both descriptive and inferential statistical analysis was done using the software. One-way Analysis of Variance (ANOVA), pairwise comparison and Pearson correlation coefficient were tested on the effect of treatments at a significance level of 0.05.

3 Results and Discussion

3.1 Grain damage assessment of Mung bean treated with seven different treatments

The number of grains perforated and percent grain perforation in Mung bean grains treated with eight different treatments is presented in Tables 2 & 3. The results show that there were no significant differences ($P \leq 0.05$) in the number of grains perforated and percent grain perforated between the treatments initially from the 15th to the 45th day after treatment application. However, after 45 days, *Acorus calamus* rhizome powder and mustard oil treated grains followed by the grains stored in super grain bag recorded significantly lesser ($P \leq 0.05$) number of grains perforated and percent grain perforated throughout the storage period. There was significant differences in the mean number of grains perforated ($P<0.001$) and percent grain perforated ($P<0.001$) between the treatments. The highest mean number of grains perforated and percent grain perforated was recorded in grains treated with *Vitex negundo* leaf powder (106.77 & 39.85 %) followed by grains treated with *Curcuma longa* powder (91.62 & 35.75 %) which were not significantly different ($P \leq 0.05$) as compared to the mean number of grains perforated (98.39) and percent grain perforated (37.80 %) in the untreated control. The lowest mean number of grains perforated and percent grain perforated was recorded in grains treated with *Acorus calamus* rhizome powder (0.46 & 0.20 %) followed by grains treated with mustard oil (1.05 & 0.47 %) and grains stored in super grain bag (5.74 & 2.49%) which were significantly lower ($P<0.001$) as compared to all other treatments.

This result is consistent with the findings of Said and Pashte (2015) who also found that Sweet flag rhizome powder treatment had significantly lower insect infestation at the end of the 10th month of storage besides having higher germination percent and seed vigour index. Similar research by Rajapakse (2006) also found a reduction in oviposition, emergence and the overall population of *Callosobruchus chinensis* with the use of *Acorus calamus* rhizome powder treatment in storage. Various vegetable oils such as sesame oil and mustard oil against *Callosobruchus maculatus* and *Bruchidius incarnates ("CABI Compendium,")* palm kernel oil and groundnut oil against *Callosobruchus maculatus* (Uddin Ii & Sanusi, 2013) were found significantly effective in suppressing various storage pests and provided a promising reduction of oviposition, deterrence and toxicity, protecting legumes in storage condition. Mustard oil was found effective for the management of cowpea weevil with minimum mean adult emergence (25) and affected grains (23.33) and higher adult mortality (40) at 90 days (Khanal, Alisha. Khadka, & Rameshwor. Pudasaini, 2020).

In an experiment by Tivana et al. (2021) who compared the effectiveness of high-density polyethylene container, super grain bags and polypropylene bag for cowpea grain storage, the use of super grain bag and polypropylene bag resulted in damaged grain of up to 13% and 52% respectively. Further, it also stated that after 4 months of storage, the super grain bag was perforated by insects, compromising its hermeticity.

Table 2. Mean number of grains perforated in store after treating with different treatments at different days after treatment

Treatment	Number of grains perforated in mung bean treated with different treatments at different days after treatment (DAT)												
	15	30	45	60	75	90	105	120	135	150	165	180	195	Mean
Acorus calamus rhizome powder	1.33	1.00	2.00	0.33^{c}	0.00 ^{c}	0.00 ^{d}	0.33 ^{d}	0.33 ^{b}	0.00 ^{c}	0.00 ^{b}	0.67 ^{d}	0.00 ^{d}	0.00 ^{d}	0.46^{d}
Mustard oil	1.33	1.33	1.67	0.67 ^{c}	1.00 ^{bc}	1.00 ^{cd}	0.67 ^{cd}	0.67 ^{b}	1.67 ^{c}	0.33 ^{b}	0.33 ^{d}	1.00 ^{d}	2.00 ^{d}	1.05^{d}
Allium sativum cloves	2.33	1.33	2.33	2.67 ^{b}	1.33 ^{bc}	3.00 ^{abc}	2.67 ^{cd}	11.33 ^{b}	61.67 ^{bc}	41.00 ^{b}	103.67 ^{c}	166.00 ^{c}	232.67 ^{ab}	48.62^{c}
Curcuma longa powder	2.67	1.00	2.33	3.67 ^{b}	2.33 ^{ab}	1.33 ^{bcd}	28.00 ^{ab}	86.33 ^{a}	125.00 ^{ab}	166.67 ^{a}	295.67 ^{a}	212.67 ^{bc}	263.33 ^{a}	91.62^{ab}
Wood ash	1.67	3.67	0.67	3.33 ^{b}	1.33 ^{bc}	1.33 ^{bcd}	18.67 ^{bc}	60.33 ^{ab}	112.00 ^{ab}	190.33 ^{a}	200.33 ^{b}	238.67 ^{bc}	198.00 ^{b}	79.26^{b}
Vitex negundo leaf powder	2.33	3.00	1.00	3.67 ^{b}	1.67 ^{b}	3.33 ^{ab}	18.33 ^{bcd}	72.00 ^{a}	191.67 ^{a}	216.33 ^{a}	327.33 ^{a}	337.67 ^{a}	209.67 ^{ab}	106.77^{a}
Super grain bag (GrainPro®)	2.67	4.33	1.67	2.67 ^{b}	1.33 ^{bc}	3.00 ^{abc}	3.33 ^{cd}	3.67 ^{b}	7.67 ^{c}	11.67 ^{b}	5.67 ^{d}	10.33 ^{d}	16.67 ^{d}	5.74^{d}
Un treated Control	2.67	2.00	3.00	5.33 ^{a}	3.33 ^{a}	4.33 ^{a}	36.33 ^{a}	101.00 ^{a}	193.67 ^{a}	160.67 ^{a}	265.00 ^{ab}	253.33^{b}	248.33 ^{ab}	98.39^{a}
P-value	0.771	0.660	0.780	<0.001	0.006	0.006	0.001	0.004	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SD	1.26	2.54	1.63	1.72	1.14	1.71	15.40	48.99	86.81	97.56	138.86	131.34	116.07	44.82

Note: Means in the column with different letters are significantly different at $P \leq 0.05$ by Duncan`s post-hoc test

Table 3. Percent Grain damaged in mung bean after treating with different treatments at different days after treatment

Treatment	Percent grain damaged (%) in mung bean treated with different treatments at different days after treatment (DAT)													
	15	30	45	60	75	90	105	120	135	150	165	180	195	Mean
Acorus calamus rhizome powder	0.72	0.43	0.87	0.15^{c}	0.00^{c}	0.00^{c}	0.14^{c}	0.14^{b}	0.00^{d}	0.00^{b}	0.28^{d}	0.00^{c}	0.00^{c}	0.20^{d}
Mustard oil	0.77	0.62	0.89	0.28^{c}	0.42^{bc}	0.43^{bc}	0.29^{c}	0.31^{b}	0.80^{d}	0.14^{b}	0.15^{d}	0.42^{c}	1.01^{c}	0.47^{d}
Allium sativum cloves	1.09	0.57	0.96	1.09^{bc}	0.57^{bc}	1.33^{ab}	1.17^{bc}	5.16^{b}	33.33^{cd}	15.15^{b}	36.03^{c}	60.11^{b}	70.58^{b}	19.73^{c}
Curcuma longa powder	1.57	0.41	0.97	1.54^{b}	0.99^{ab}	0.57^{bc}	10.44^{a}	38.34^{a}	59.21^{abc}	50.64^{a}	82.55^{a}	71.75^{ab}	94.07^{a}	35.75^{a}
Wood ash	0.76	1.61	0.27	1.43^{b}	0.56^{bc}	0.57^{bc}	7.13^{ab}	23.07^{ab}	50.30^{bc}	58.51^{a}	60.83^{b}	69.09^{ab}	58.42^{b}	29.64^{b}
Vitex negundo leaf powder	1.27	1.23	0.41	1.60^{b}	0.72^{b}	1.40^{ab}	7.38^{ab}	27.71^{ab}	80.27^{ab}	62.92^{a}	90.06^{a}	84.77^{a}	92.35^{a}	39.85^{a}
Super grain bag (GrainPro®)	1.36	2.12	0.69	1.13^{b}	0.57^{bc}	1.3^{ab}	1.49^{bc}	1.72^{b}	3.50^{d}	4.54^{b}	2.33^{d}	4.20^{c}	6.82^{c}	2.49^{d}
Un treated Control	1.36	0.85	1.21	2.30^{a}	1.40^{a}	1.77^{a}	13.25^{a}	4.91^{a}	89.09^{a}	52.22^{a}	85.54^{a}	74.72^{ab}	91.09^{a}	37.80^{a}
P-value	0.765	0.555	0.799	0.000	0.006	0.008	0.001	0.010	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SD	0.70	1.13	0.69	0.75	0.48	0.72	5.68	21.25	38.28	29.12	39.16	36.60	41.50	16.91

Note: Means in the column with different letters are significantly different at $P \leq 0.05$ by Duncan`s post-hoc test

3.2 Insect Perforation Index (IPI)

There was highly significant difference (P<0.001, SD=22.09) observed on the mean Insect Perforation Index (IPI) between the treatments. The lowest IPI was recorded in grains treated with *Acorus calamus* rhizome powder (0.54) followed by mustard oil treatment (1.24) and grains stored in super grain bag (6.13) which were significantly lower (P<0.001) as compared to all other treatments. The highest IPI was recorded in grains treated with *Vitex negundo* leaf powder (51.29) which was higher than 50 indicating an index of negative protectability (Ileke, Idoko, Ojo, & Adesina, 2020). The mean IPI in mung bean grains treated with *Acorus calamus* rhizome powder reached zero after 75 days of treatment application and remained below 1 till the end of the storage period.

Table 4. Insect perforation index in grains treated with different treatments

Treatment	Insect perforation index (%) in mung bean treated with different treatments at different days after treatment (DAT)													
	15	30	45	60	75	90	105	120	135	150	165	180	195	Mean
Acorus calamus rhizome powder	36.50	33.68	44.94	5.90^{b}	0.00^{c}	0.00^{d}	0.76^{b}	0.43^{b}	0.00^{c}	0.00^{c}	0.36^{d}	0.00^{c}	0.00^{c}	0.54^{d}
Mustard oil	38.50	22.96	44.50	9.26^{b}	20.27^{bc}	18.46^{cd}	3.19^{b}	0.42^{b}	0.89^{c}	0.21^{c}	0.19^{d}	0.57^{c}	1.14^{c}	1.24^{d}
Allium sativum cloves	43.69	22.41	43.73	32.12^{a}	28.7^{ab}	42.79^{abc}	10.28^{b}	13.45^{b}	23.28^{b}	22.14^{b}	29.62^{c}	44.24^{b}	43.03^{ab}	34.06^{c}
Curcuma longa powder	51.75	19.49	37.66	40.03^{a}	41.33^{ab}	19.63^{bcd}	44.3^{a}	45.68^{a}	39.96^{ab}	48.65^{a}	48.67^{a}	48.77^{ab}	50.80^{a}	48.56^{ab}
Wood ash	36.81	54.97	22.17	37.93^{a}	26.56^{ab}	15.08^{d}	37.58^{a}	36.11^{a}	33.07^{ab}	53.50^{a}	41.10^{b}	47.98^{ab}	38.99^{b}	43.83^{b}
Vitex negundo leaf powder	36.84	37.66	30.85	40.46^{a}	31.22^{ab}	44.6^{ab}	38.31^{a}	42.07^{a}	47.20^{a}	52.90^{a}	51.33^{a}	53.17^{a}	50.32^{a}	51.29^{a}
Super grain bag (GrainPro®)	47.36	63.01	27.81	32.97^{a}	27.92^{ab}	42.11^{abc}	11.92^{b}	3.63^{b}	3.52^{c}	8.12^{c}	2.72^{d}	5.33^{c}	6.38^{c}	6.13^{d}
Un treated Control	50.02	49.93	49.94	50.00^{a}	50.00^{a}	49.97^{a}	50.00^{a}	50.00^{a}	50.00^{a}	50.00^{a}	50.00^{a}	50.00^{ab}	50.00^{a}	50.00^{ab}
P-value	0.963	0.515	0.945	0.000	0.008	0.002	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SD	19.18	29.26	27.60	16.86	17.47	20.53	22.24	23.12	21.74	23.62	22.51	23.55	22.54	22.09

Note: Means in the column with different letters are significantly different at $P \leq 0.05$ by Duncan`s post-hoc test

3.3 Correlation on number of adult insects against percentage of grains perforated

Person correlation coefficient was conducted among quantitative parameters (Table 5) to explore on the number of adult insects against percentage of grains perforated. It was observed that there was highly significant positive correlation (*r*=0.939, *P*<0.001) on number of adult insect with percentage of grains perforated. This reveals that the percentage of grains perforated will increase with the increase in number of adult cowpea weevils. The number of cow pea weevil remained zero till 60th day after treatment application and increased exponentially till 150th day and declined as shown in (Figure 1).

Table 5. Correlation on number of adult insect against percentage of grains perforated

Characters	No. of adult insects	% grains perforated	Insect Perforation index (IPI)
No. of adult insects	1	0.939**	0.937**
% grains perforated	0.939**	1	0.987**
Insect Perforation index (IPI)	0.937**	0.987**	1

*Correlation is significant at the 0.05 level (2-tailed)

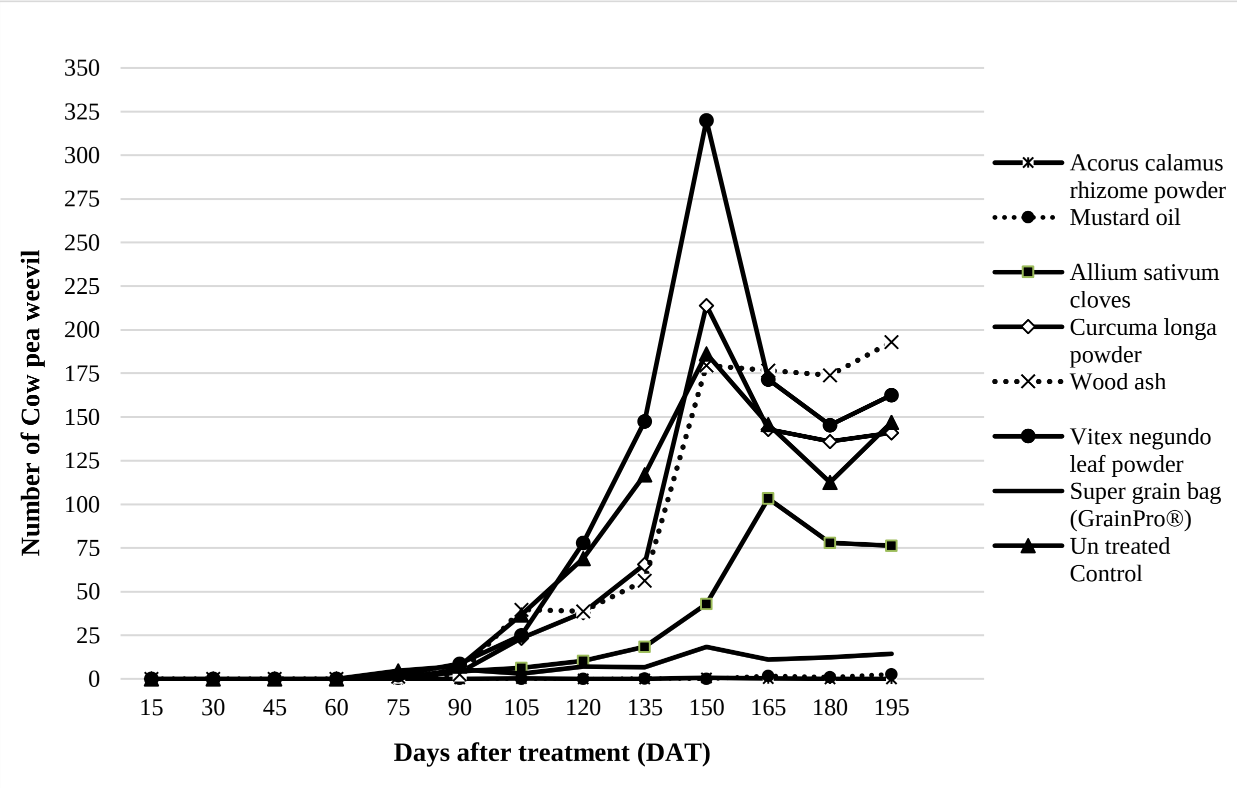

Figure 1. Number of adult Cow pea weevils recorded in grains treated with different treatments at 15 days interval

3.4 Seed germination percent and seed vigour index

The seed germination test result shows a highly significant difference in the mean seed germination percentage among all the treatments from the statistical analysis ($P<0.013$, $SD=11.16$) (Table 6). From pair wise comparison between treatments, highest germination percentage was in wood ash (98.67%) while the lowest was in super grain bag (70.67%). However, there were no significant differences ($P=0.284$) in seed vigour index among the treatments. The highest mean seed vigour index was in wood ash treatment (12.75) and lowest in super grain bag (9.46). So this study reveals that all the eight treatments do not have any significant effect ($P=0.284$) on the seed vigour index. However, seed germination percentage in grains treated with Wood ash (98.67%), Mustard oil (96.0%), *Acorus calamus* rhizome powder (95.0%), Garlic (95.0%) and untreated control (94.0%) were significantly higher ($P=0.013$) than germination percentage of grains stored in super grain bag (70.67%). Although the percent grain perforated (2.49%) and insect perforation index (6.13) was significantly lower in grains stored in super grain bag, significantly lower seed germination percentage (70.67%) indicates that super grain bag can be used preferably for grain storage but not for seed storage purpose.

Table 6. Percentage of seed germination and seed vigour index

Treatment	Seed germination (%)	Seed vigour index
Sweet flag rhizome powder (*Acorus calamus*)	95.00 [a]	9.76
Mustard oil	96.00 [a]	10.95
Garlic cloves (*Allium sativum*)	95.00 [a]	12.31
Turmeric rhizome powder (*Curcuma longa*)	90.67 [ab]	10.29
Wood ash	98.67 [a]	12.75
Chinese chaste tree leaves (*Vitex negundo*)	91.00 [ab]	11.67
Super grain bag	70.67 [b]	9.46
Control	94 .00[a]	11.01
CV (%)	8.32	15.65
p-value	0.013	0.284

4 Conclusion

From this study, it was found that the lowest percent grain perforated and insect perforation index was found in grains treated with *Acorus calamus* rhizome powder followed by mustard oil and grains stored in super grain bag as compared to other treatments. The lowest number of adult weevils was in grains treated with *Acorus calamus* rhizome powder followed by mustard oil and super grain bag throughout the storage period. The highest percent grain perforated was recorded in grains treated with *Vitex negundo* leaf powder followed by *Curcuma longa* powder which were found ineffective in managing cowpea weevil. However, the highest germination percentage was found in wood ash followed by mustard oil, *Acorus calamus* and garlic-treated grains while the lowest was in super grain bag. Therefore, *Acorus calamus* rhizome powder and mustard oil were found to be effective in managing cowpea weevil without affecting seed germination and vigour. Although the super grain bag was also found to be effective against cowpea weevil, it can be recommended for storing grains for consumption only but not for seed purposes. Further studies need to be conducted on the level of toxicity of the treated grains for human consumption.

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Evaluation and Selection of Open-pollinated Tomato (*Solanum lycopersicum* L.) Entries for Adaptation under Temperate Agroecological Conditions of Bhutan

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ABSTRACT

Lack of high-yielding climate-resilient varieties and, frequent pest and disease incidences are the major issues in tomato production in Bhutan. The National Centre of Organic Agriculture, Yusipang, introduced 40 open-pollinated entries between 2020 and 2021. With an objective to evaluate and select the most desired tomato varieties for commercial cultivation in Bhutan, seven tomato entries were prescreened and selected using combined scoring of the total votes from the Participatory Varietal Selection and yield of entries in 2021. The Randomized Block Design with eight entry treatments and three replications with Ratan as standard check was employed to evaluate their yield and yield parameters; fruit quality; and tolerance to pests and diseases in 2022. ANOVA followed by Tukey HSD test for mean separation was employed at p-value at P<0.05. The result showed that AVTO1954 produced a significantly higher yield (29.8 tons/acre) compared to AVTO1910 but not significantly different from AVTO1702 (28.5 tons/acre) and AVTO1907 (28.4tons /acre). Although Roma (check) produced the highest total number of fruits per plant (110), it produced the lowest number of marketable fruits per plant (7) compared to all other entries, while the plant height did not show any statistically significant differences between different treatment entries. Two entries with the lowest disease incidence were AVTO1702 and AVTO1954, while Roma was infested with blight and powdery mildew at 27% and 45% respectively. The study recommends the release of three entries viz-a-viz AVTO1954, AVTO1907 and AVTO1702 and similar research in other agroecological zones of Bhutan to identify appropriate varieties in their zones.

Keywords: *Tomato; Germplasms; Selection; Participatory Varietal Selection*

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1 Introduction

Tomato (*Solanum Lycopersicum* L.) is one of the most widely cultivated and consumed vegetable crops globally (Bihon et al., 2022). It is generally consumed fresh, cooked, or processed into various products such as ketchup and paste (Rawal et al., 2017). It is rich in minerals, ascorbic acids, organic sugars and fibres including vitamins B and C (Ali et al., 2016). The lycopene in tomatoes is reported to be one of the most potent antioxidants, which plays an important role in the prevention of certain forms of cancer and cardiovascular disease (Agarwal & Rao, 2000).

Tomato is one of the priority crops identified by the Department of Agriculture, Ministry of Agriculture and Forests (DoA, 2017). Although production has gradually increased over the years with about a 10 percent jump from 2016 to 2021, domestic supply is still too low to meet the country's demand. The total cultivated area covered by tomatoes was 229 acres with a total production of 289 tons in the year 2021 (NSB, 2022). Consequently, in the same year, Bhutan imported 4296.63 tons with a corresponding value of about Nu. 150 million to fill in the supply gap making it one of the highest imported vegetables in the country (MoF, 2021).

Bhutan has a high potential to enhance tomato production to meet domestic demand owing to its diverse agroecological conditions. In Bhutan, tomatoes are cultivated under protected structures to enhance production by protecting the crops from pests and diseases; compensate for low temperatures in high altitudes (Sotelo-Cardona, Lin, & Srinivasan, 2021). However, tomato production in Bhutan is constrained by a lack of high-yielding varieties, frequent pest and disease outbreaks and unorganized marketing channels. The major disease affecting tomato cultivation in Bhutan is a fungal disease called blight (NPPC, 2017). The production season of tomatoes coincides with the rainy season which makes it conducive for fungus to thrive and affect the tomato plants. The problem is more severe in organically managed farms due to no or limited organic inputs available to control the blight disease (Singh et al., 2021). Further, Bhutan has only two officially released varieties namely Ratan and Roma which are degenerated and become highly susceptible to blight with shorter fruit shelf life and low yield as it was released in 2002 (DoA, 2017). As an alternative effort to increase tomato production in the country, hybrid tomatoes namely *Cosmic* and *Garv* were tested for adaptability and performance and are being promoted. However, like elsewhere, the sustainability, as well as the cost of such imported hybrids, remains a serious concern (Kutka, 2011).

It has therefore become necessary for Bhutan to initiate proper research to select sustainable and economical tomato varieties with desired traits for promotion in the farmer's field. One such method is through the introduction and evaluation of open-pollinated varieties which are sustainable due to their ability to produce seeds year after year with improved adaptation (Gotame, Gautam, Ghimire, & Shrestha, 2021). They are also considered to be an important source of plant materials for future breeding programs. Furthermore, open-pollinated varieties are preferred for organic farming due to their high adaptation and climate-resilient capabilities. The main objective of the current study was to evaluate and select the best varieties with desirable traits of high yields, disease resistance and adaptability to Bhutan's climatic conditions.

2 Materials and Method

2.1 Study location and details

The study was conducted at the National Center for Organic Agriculture, Yusipang (NCOA) which is in the cool temperate agroecological zone (latitude of 27 °27'50" N and longitude of 89 °42 '25 "E) at an elevation of 2700 meters above sea level. It receives an annual rainfall ranging between 50 mm to 650mm with an annual mean temperature of about 12.5 °C. The study was conducted over a period of three years from March 2020 to September 2022. In addition, an evaluation was conducted at two locations (Ramthangkha and Bjemina villages of Paro and Thimphu Dzongkhag respectively) to understand the performance of selected varieties in the farmer's field.

2.2 Tomato entries

A total of 40 tomato entries were introduced for evaluation and selection during the study duration from the World Vegetable Centre based in Taiwan. In the first year (2020), 23 entries were evaluated under open field. However, almost all the entries except six entries from the open field cultivation were lost to blight disease due to which the studies in the following years were conducted under the greenhouse condition. In the second year, six entries rescued from the first-year cultivation trials along with 17 new entries were cultivated on a standard bed size of 2 m x 1 m without any replication inside greenhouses.

2.3 Pre-screening and ranking of entries

The data from the Participatory Varietal Selection (PVS) method and the yield from the experiment were used to perform the pre-screening of the most desired entries. The PVS was done involving farmers from Yusipang and Hongtsho communities who have some experience in tomato farming and marketing. The ranking of entries was done by ranking the sum of standardized scores calculated by addition of standardized values of total votes and yield (Table 2). The rows values from PVS and yield (Table 1) were normalized using the equation (Eq. i). The top seven entries were selected for further evaluation following replicated trial in the final year (2022).

$$X_{Scaled} = \frac{X - X_{min}}{X_{max} - X_{min}} \quad \text{Eq. i}$$

Whereas,

X_{Scaled} is standardized values

X is the row values of total votes/yield

X_{min} is the minimum values of array of row values

X_{max} is the maximum value of array of row values

Table 1. Raw vote and yield data from PVS and field trial

Entry number	Growth habit	Vote Count (Male)	Vote Count (Female)	Total Votes	Yield (Tons/Acre)
AVTO1954	D	96	52	148	10.06
AVTO1907	D	50	10	60	16.72
AVTO1003	D	57	15	72	14.90
AVTO1705	D	38	45	83	13.00
AVTO1911	D	42	27	69	13.29
AVTO1702	D	75	48	123	5.87
AVTO1910	D	61	39	100	7.20
AVTO1909	D	39	25	64	9.95
AVTO1919	D	10	9	19	13.22
AVTO1008	D	11	5	16	10.79
AVTO1912	SD	8	7	15	10.61
AVTO1921	SD	10	12	22	7.95
AVTO1828	ID	0	0	0	10.06
AVTO1915	SD	10	8	18	7.64
AVTO1903	D	13	7	20	7.18
AVTO1010	D	15	4	19	6.20
AVTO1315	NA	0	0	0	7.33
AVTO1913	SD	8	6	14	4.98
AVTO1706	SD	0	0	0	4.09
AVTO1914	SD	0	0	0	3.78
AVTO1288	NA	0	0	0	2.69

AVTO0301	SD	0	0	0	1.58
AVTO1829	ID	0	0	0	0.00

D = Determinate; SD = Semi determinate; ID = Indeterminate; NA = Not Available

Table 2. Rank, normalized scores for total votes, yield and sum of normalized values of tomato entries

Entry number	Normalized votes	Normalized yield	Sum of score	Rank
AVTO1954	1.00	0.60	1.60	1
AVTO1907	0.41	1.00	1.41	2
AVTO1003	0.49	0.89	1.38	3
AVTO1705	0.56	0.78	1.34	4
AVTO1911	0.47	0.79	1.26	5
AVTO1702	0.83	0.35	1.18	6
AVTO1910	0.68	0.43	1.11	7
AVTO1909	0.43	0.60	1.03	8
AVTO1919	0.13	0.79	0.92	9
AVTO1008	0.11	0.65	0.75	10
AVTO1912	0.10	0.63	0.74	11
AVTO1921	0.15	0.48	0.62	12
AVTO1828	0.00	0.60	0.60	13
AVTO1915	0.12	0.46	0.58	14
AVTO1903	0.14	0.43	0.56	15
AVTO1010	0.13	0.37	0.50	16
AVTO1315	0.00	0.44	0.44	17
AVTO1913	0.09	0.30	0.39	18
AVTO1706	0.00	0.24	0.24	19
AVTO1914	0.00	0.23	0.23	20
AVTO1288	0.00	0.16	0.16	21
AVTO0301	0.00	0.09	0.09	22
AVTO1829	0.00	0.00	0.00	23

2.4 Nursery management

Seeds were sown in the March under polytunnels to protect the juvenile seedlings from insect infestation and other external factors such as wind, rain, etc. Nursery beds of dimension 2 m x 1 m were prepared by ploughing the land using a power tiller followed by mixing the fine soil with organic manure. Brief irrigation was done after sowing to facilitate the germination of the seeds. Seedlings were hardened for a week when the seedling attained about 8 cm to 10 cm in height with at least five number of leaves before transferring to the greenhouses.

2.5 Study design

The study was conducted under greenhouses of 20 m x 5 m dimensions. The trial was laid out in a Randomized Complete Block Design (RCBD) with 8 treatments of tomato entries which were replicated 3 times. The 8 treatment plots of raised beds with the dimensions of 2.5 m x 1.2 m were prepared with a spacing of 0.30 m in between to facilitate intercultural operations. All the treatment plots were then mulched with black mulching plastics followed by drilling 24 numbers of holes in each plot at a plant-to-plant distance and row-to-row distance of 0.40 m and 0.60 m respectively between rows. Tomato varieties namely Roma which is an open-pollinated and released variety was used as a standard check.

2.6 On-farm trial

The on-farm trials were conducted in two districts namely Paro and Thimphu to understand the performance of selected entries under farmer's management conditions. The treatment plots were prepared with the same dimensions, mulching materials, and plant-to-plant and row-to-row distance as that followed in the on-station trial. However, it was not possible to establish the desired replicated trials in the farmer's field due to limited polyhouses and farmers' preferences for growing other crops such as chili during the season.

2.7 Data Collection

A standard data collection format was adopted from Hanson et al. (2011). Data on yield and yield parameters, disease resistance, and their fruit characteristics were collected from five randomly selected plants from each treatment plot. Yield and yield parameters included the number of total fruits, number of marketable fruits, number of non-marketable fruits, the weight of marketable fruits (g), and weight of non-marketable fruits (g). Plant height was measured using a measuring tape from five sample plants from each treatment plot during the last harvest. The growth habit (determinate, indeterminate, semi-determinate) of the tomato entries was recorded consistently during the study period. The incidences of disease and pests such as late blight, powdery mildew, yellow leaf curl disease, bacterial wilt, fruit borer and collar rot were recorded on fortnightly basis. The disease and pest incidence in the treatment plot was calculated using the formula in the equation (Eq. ii).

$$PDI\ (\%) = \frac{NIPs}{TNP}\ x\ 100 \qquad \text{Eq. ii}$$

Whereas,

PDI (%) is the pest and disease incidence in a treatment plot in percentage

NIPs is the total number of infested or infected plants in a treatment plot

TNP is the total number of plants in a treatment plot

Fruit characteristics such as fruit weight (g), fruit diameter (mm) and fruit length (mm) were measured from 10 randomly selected fruits at each harvest. While segregating marketable and non-marketable fruits, the following conditions were considered.

a) Fruits free from blemish and defects which weighed more than 30 grams with an equatorial diameter of more than 35 mm were categorized as "marketable fruits".
b) Fruits which weighed less than 30 grams with a diameter of less than 35 mm and the ones with defects or blemishes were categorized as "non-marketable fruits".

2.8 Data analysis

Raw data collected were entered, cleaned, and preprocessed in Microsoft Excel 365. Data were statistically analyzed in R statistical software version 4.2.2 (R Core Team, 2022) using a one-way analysis of variance (ANOVA) followed by mean separation using Tukey Honest Significance Difference test. The alpha value was set at P = 0.05 to detect statistically significant differences between all the comparisons made in this article. The R-packages such as 'tidyverse' version 1.3.2 (Wickham et al., 2019), 'ggstatsplot' version 0.9.5 (Patil, 2021), 'dlookr' version 0.6.0 (Ryu, 2022), and 'car' version 3.1.1 (Fox & Weisberg, 2019) were used for data wrangling, cleaning and analysis. The graphical representations of the statistical data were prepared using r-package 'ggplot2' version 3.3.6 (Wickham, 2016).

3 Results and Discussion

3.1 Yield of tomato entries

It is evident from the result (Figure 1) that AVTO1954 produced a significantly higher yield (29.8 tons/acre) compared to AVTO1910 (9.91 tons/acre) while it did not show any statistically significant difference with other tomato entries including the check variety. The yields were not significantly different between entries AVTO1702 (28.50 tons/acre), AVTO1907 (28.4 tons/acre), AVTO1705 (27.50 tons/acre), AVTO1003 (22.10 tons/acre), AVTO1911 (19.20 tons/acre) and Roma (20.30 tons/acre). On the other hand, yields of entries AVTO1003, AVTO1911, Roma and AVTO1910 were statistically similar. Our results also match with a report from the World Vegetable Center (World Veg, 2022) on improved tomato lines according to which the AVTO1954 is considered blight resistance and most suitable for cool and wet conditions. The low total yield in AVTO1910 is due to weak plants and poor adaptability leading to high mortality after transplanting and throughout the growing season.

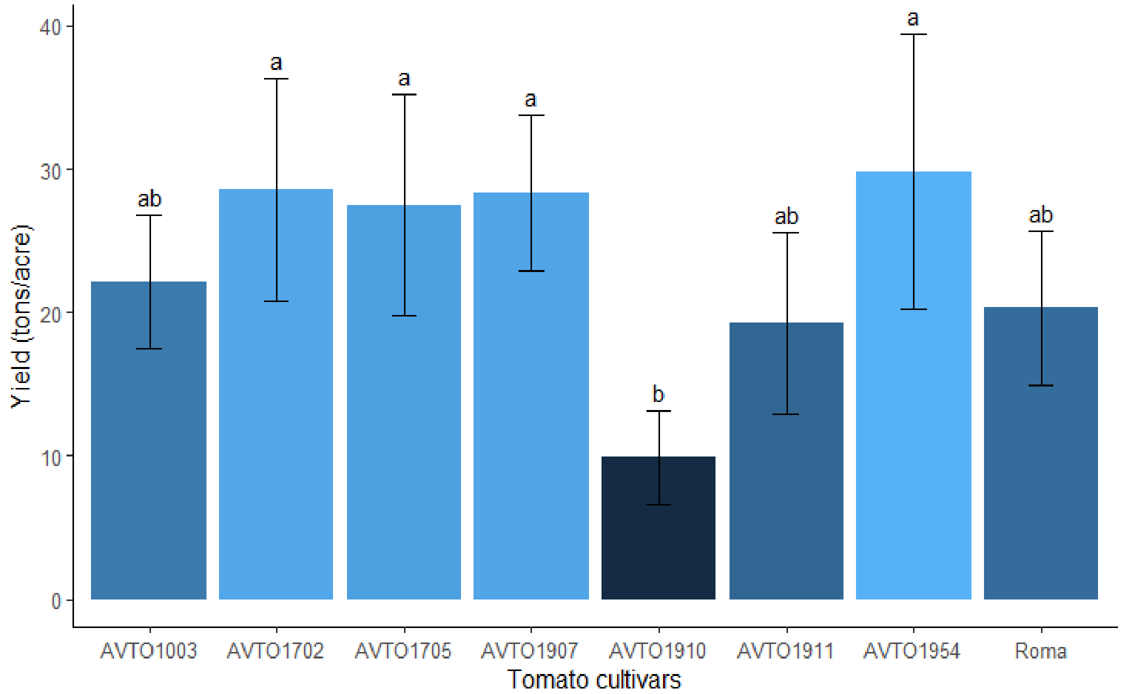

Figure 1. Yield (Tons/Acre) of tomato entries. Different lower-case letters indicate statistically significant differences following Tukey's HSD post hoc test at $P<0.05$

3.2 Total number of fruits per plant

The result (Figure 2) revealed that the total number of fruits per plant was highest in Roma (110) which was significantly higher than AVTO1911 (51) and AVTO1910 (31) but not significantly different from AVTO1907 (89), AVTO1954 (89), AVTO1702 (87), AVTO1705 (84) and AVTO1003 (59). The total number of fruits produced by AVTO1911 was not significantly different from that of AVTO1910, AVTO1003 and AVTO1705. The result also clearly indicated that the number of fruits produced by AVTO1907, AVTO1954, AVTO1702, AVTO1705, AVTO1003 and AVTO1911 was not significantly different from each other. The result shows that Roma may be a very good fruit bearer, but it gives poor fruit quality which may not be profitable to our farmers (Figure 43, 4). The result also indicated that the variety Roma is the least productive under protected conditions.

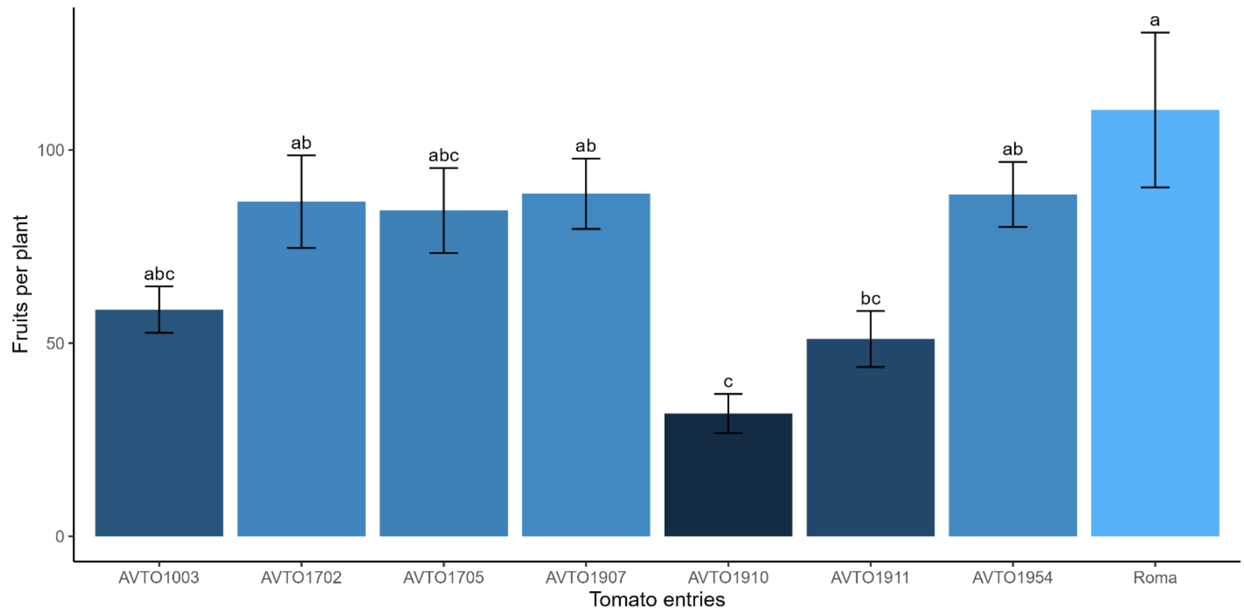

Figure 2. Total fruits/plant of different tomato entries. Different lower-case letters indicate statistically significant differences following Tukey's HSD post hoc test at $P<0.05$

3.3 Number of marketable fruits per plant

As shown in (Figure 3), there were highly significant differences in terms of the number of marketable fruits of AVTO1954 (39) and AVTO1907 (35) compared to the check variety Roma (7) at $P<0.05$, however, they were not significantly different to that of AVTO1705 (33), AVTO1702 (29), AVTO1911 (28), AVTO1003 (27), and AVTO1910 (18). Further, the total number of marketable fruits per plant from AVTO1705, AVTO1702, AVTO1911, AVTO1003, and AVTO1910 was not significantly different from each other. The result confirms that the check variety Roma is degenerated and no more suitable for commercial cultivation.

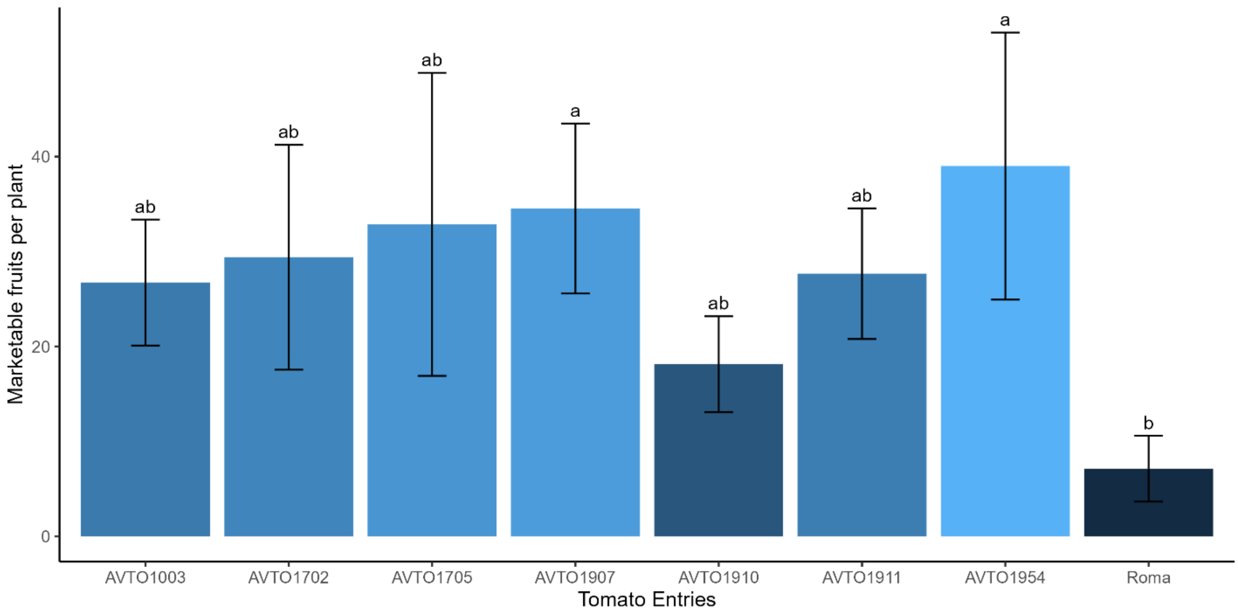

Figure 3. Number of marketable fruits/ per plant. Different lower-case letters indicate statistically significant differences following Tukey's HSD post hoc test at $P<0.05$

3.4 Number of non-marketable fruits per plant

The result (Figure 4) of the total number of non-marketable fruits per plant produced by different tomato entries shows that the check variety Roma (103) produced a highly significant number of non-marketable fruits compared to all other tomato entries except AVTO1702. In contrast, the total number of non-marketable fruits per plant from AVTO1702 (57), AVTO1907 (54), AVTO1705 (51), AVTO1954 (49), AVTO1003 (32), AVTO1911 (23) and AVTO1910 (13) were not statistically different. The result consistently confirmed that the performance of new entries of tomatoes is better compared to the check variety.

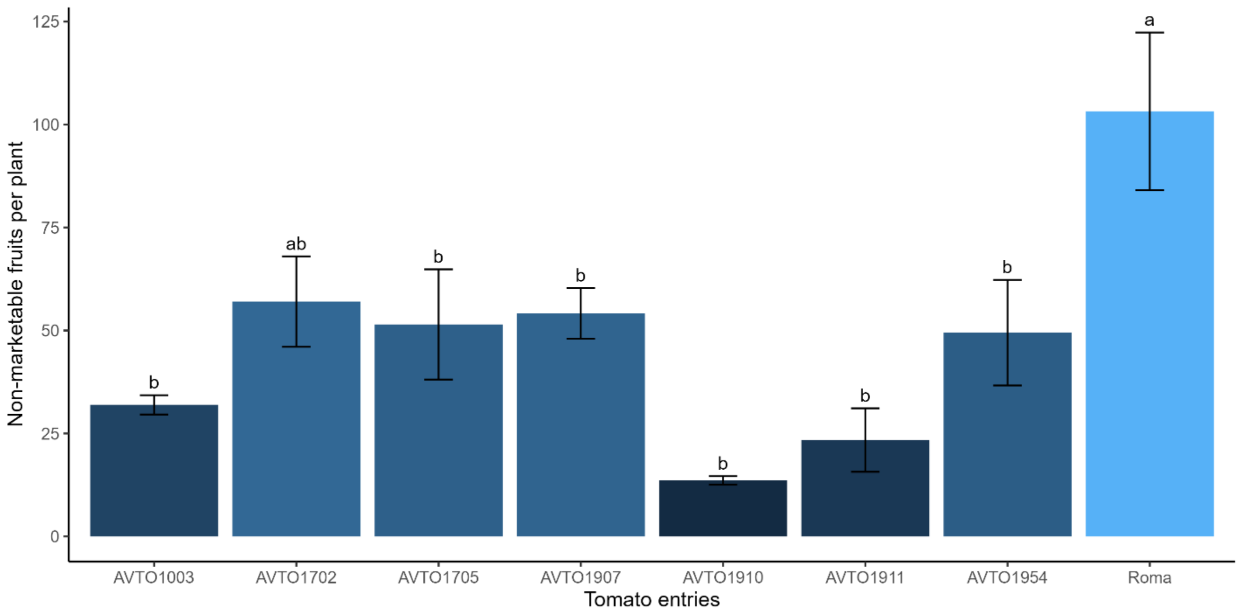

Figure 4. Total number of non-marketable fruits per plant of tomato entries. Different lower-case letters indicate statistically significant differences following Tukey's HSD post hoc test at P<0.05

3.5 Fruit characteristics of tomato entries

The mean values of fruit length (mm), fruit diameter (cm), fruit weight (g) and corresponding fruit shapes are presented in (Table 3). It revealed that the tomato entry AVTO1907 produced a significantly longer fruit length compared to all other entries with the shortest fruit length from Roma. Further, the fruit length of all the entries were found to be significantly different from each other except between AVTO1702 and AVTO1910; AVTO1910 and AVTO1705.

The AVTO1954 and Roma obtained fruits with the widest and narrowest diameter respectively. The fruit diameter of AVTO1954 was significantly different from AVTO1003, AVTO1907 and AVTO1910, and not from AVTO1702, AVTO1705 and AVTO1911 while the Roma obtained fruits with significantly narrow widths compared to all other entries. However, tomato entries (AVTO1702, AVTO1705, AVTO1907, AVTO1910, and AVTO1911) obtained statistically similar fruit width.

The fruits from AVTO1911 were significantly heavier compared to that from AVTO1003, AVTO1705, AVTO1954 and Roma, however, they were of statistically similar weight with fruits from AVTO1702, AVTO1907 and AVTO1910. The fruit of AVTO1954 was significantly heavier than that of AVTO1705 and Roma. On the contrary, Roma produced significantly lightest fruits compared to all other entries under study.

Table 3. Mean (SE) of fruit length, fruit width, fruit weight and fruit shape of different tomato entries

Treatment	Mean fruit length (mm)	Mean fruit width (mm)	Mean fruit weight (g)	Fruit shape
AVTO1003	57.09(1.02)^{b}	39.14(0.09)^{c}	52.63(2.2)^{b}	Oblong
AVTO1702	53.1(0.63)^{d}	45.16(0.75)^{ab}	64.93(0.55)^{ab}	Oblong-blocky
AVTO1705	51.23(0.39)^{e}	45.3(2.87)^{ab}	52.33(2)^{c}	Round, pointed tip
AVTO1907	58.88(0.22) ^{a}	42.89(0.13)^{b}	60.03(1.17)^{ab}	Oblong
AVTO1910	51.79(1.15)^{de}	42.77(0.44)^{b}	58.7(4.37)^{ab}	Oblong
AVTO1911	55.08(0.11)^{c}	44.55(0.51)^{ab}	68.77(0.45)^{a}	Blocky to oblong
AVTO1954	42.45(0.06)^{f}	46.39(0.38)^{a}	53(2.96)^{b}	Blocky
Roma (check)	30.15(0.13)^{g}	34.1(1)^{d}	29.87(5.23)^{d}	Oblong
F-Statistics	*712*	*38.27*	*38.27*	
P-value	*<0.001*	*<0.001*	*<0.001*	

Different lower-case letters in the superscript indicate statistically significant differences following Tukey's HSD post hoc test at P<0.05

3.6 Plant height of tomato entries

The mean plant height of different tomato entries measured during the last harvest is subjected to the Analysis of Variance test to see if there is a statistical difference in the height of different entries tested in this study (*result not shown*). Although there is no significant difference in the plant height of all the entries, the tallest plants were obtained from AVTO1911 (103.5 cm) followed by AVTO1003 (102.4 cm) and AVTO1705 (102 cm), AVTO1954 (95.20 cm), AVTO1702 (94.50 cm), AVTO1907 (93.10 cm), Roma (92.90 cm). The shortest plants were obtained from AVTO1910 with a mean plant height of 73.10 cm.

3.7 Pests and disease incidence

As the experiment was conducted under greenhouse conditions, the incidences of pests and diseases were very low to cause any significant reduction in crop growth and yield. The minor incidences of powdery mildew and blight diseases observed were recorded and the result is presented in table (Table 4). The result shows that the check variety Roma had a comparatively higher level of disease infection with the severity score of powdery milder and blight of 45% and 27% respectively, while other entries some incidences of diseases with no sign of blight incidence in AVTO1702 and AVTO1954.

Table 4. Pest and disease incidence (Blight and powdery mildew)

Treatments	Blight				Powdery mildew			
	TNP	NHP	NIP	PDI (%)	TNP	THP	TIP	PDI (%)
AVTO1003	24	20	2	10	24	20	4	20
AVTO1705	24	21	3	14	24	21	4	19
AVTO1907	24	24	2	8	24	24	6	25
AVTO1702	22	20	0	0	22	20	6	30
AVTO1911	19	19	3	16	19	19	6	32
AVTO1954	23	20	0	0	23	20	8	30
AVTO1910	17	16	3	18	17	16	4	25
Roma	20	18	5	27	20	18	9	45

3.8 Yields (tons/acre) of tomato entries from the on-farm and on-station trial

The mean yield (tons/acre) of introduced and selected tomato entries from all three study sites are presented in figure (Figure 5). Overall, all the tomato entries yielded the lowest at Bjimina while yields obtained from Ramthangkha and Yusipang were similar for all the entries except AVTO1910. The result from Ramthangkha showed that AVTO1954 (39 tons/acre) yielded the highest followed by AVTO1702 (29 tons/acre), AVTO1907 (28.90 tons/acre), AVTO1003 (27.10 tons/acre), AVTO1705 (25.50 tons/acre), AVTO1911 (23 tons/acre), while AVTO1910 obtained the lowest yield of 20 tons/acre. Similarly, at Bjimina, AVTO1705, AVTO1702 and AVTO1954 obtained the highest mean yield of 11 tons/acre followed by AVTO1910 (10 tons/acre), AVTO1003 and AVTO1907 with the same yield of 9 tons/acre, while AVTO1911 produced the lowest yield of 6 tons/acre. Furthermore, results from Yusipang showed that AVTO1954 gave the highest yield of 29.8 tons/acre followed by AVTO1702 (28.5 tons/acre), AVTO1907 (28.4 tons/acre), AVTO1705 (27.5 tons/acre), AVTO1003 (22 tons/acre), AVTO1911 (19.2 tons/acre) and lowest yield from AVTO1910 with 10 tons/acre. The consistently low yield in tomato entries at Bjimina was due to taking record of only the first three harvests by the farmers after which the crops were uprooted for leguminous vegetables and Cole crops. The findings confirm that the three entries (AVTO1954, AVTO 1705 and AVTO1907) have consistently shown higher yields in all three locations which can be identified for further cultivation on a mass scale by tomato farmers.

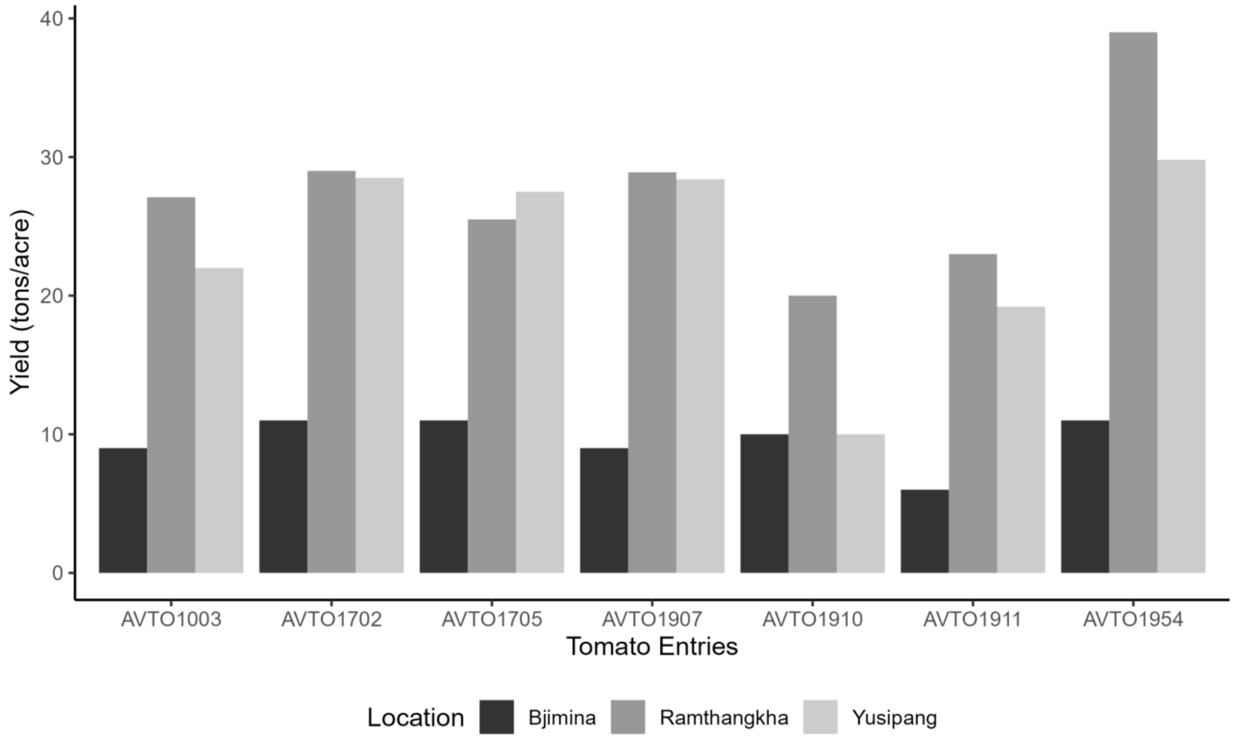

Figure 5. Mean yield (tons/acre) of AVRDC tomato entries at three study sites

4 Conclusion

The present investigation and research revealed that the tomato varieties introduced by the World Vegetable Centre, Taiwan are superior in terms of marketable yield, fruit quality and disease resistance compared to the standard check (Roma). The overall yield of AVTO1954, AVTO1702, AVTO1907 and AVTO1705 were significantly higher compared to AVTO1910. However, all the new entries produced a significantly higher number of marketable fruits compared to the check variety. Although the total number of fruits of standard check (Roma) was significantly higher compared to AVTO1910 and AVTO1911, no statistical difference was observed with other entries. However, Roma obtained the lowest significant number of marketable fruits compared to AVTO1954, and a significantly higher number of non-marketable fruits compared to all other entries. Therefore, we can conclude that for the commercial promotion of varieties, it is not the total yield that is important but the total number of marketable fruits which is more critical. In addition, tomato entries viz-a-viz AVTO1954, AVTO1702 and AVTO1907 could be selected for promotion and commercial production in temperate conditions of Bhutan. The result from the on-farm trials also confirms our conclusion.

We recommend that proper research focusing on pest and disease resistance be conducted with these entries to draw a scientifically concrete conclusion on their response to major tomato pests and diseases. Furthermore, similar research in the other agroecological zones in Bhutan is recommended to select appropriate entries for respective zones.

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Benefits of using Plastic Crate compared to Poly Sack during Transportation of Vegetables

Karma Dorji[3], Kinley Wangmo[4], Thinley Wangdi[5]

ABSTRACT

Broccoli, cabbage and cauliflower are some of the common vegetables that are grown and traded in Bhutan. It is cultivated in one part of the country and then transported to urban areas of the country for marketing. Poly sacks are commonly used for packing and transporting these vegetables. Plastic crates are recommended and promoted for use in packing and transporting vegetables but there is no study that has been conducted to prove their benefits. The aim of this study was to assess the transportation damage and economic benefit of using plastic crates during the transportation of vegetables compared to that of the poly sack. Broccoli, cabbage and cauliflower were harvested in the late afternoon from Chali Gewog, sorted, packed and transported to the market in Bumthang town in the same evening. At the market, vegetables were assessed for transportation damage and physiological loss of weight was recorded. Broccoli, cabbage and cauliflower packed in poly packs reported transportation damage of 8.75, 8.33 and 12.50 heads against 0.96, 0.44 and 0.41 percent for the plastic crate-packed vegetable samples with significant differences at P≤0.05. Poly-packed samples had significantly higher PLW of 2.02 kg, 2.83 kg and 3.40 kg compared to much lower PLW of 0.21 kg, 0.24 kg and 0.36 kg for broccoli, cabbage and cauliflower, respectively. Among the vegetables packed in poly sacks, transportation damage was significantly higher for cauliflower at 12.5 heads compared to 8.75 and 8.33 heads for broccoli and cabbage. Transportation damage was minimal and not significantly different between the three vegetables for plastic crates sample. Harvesting damage of 0.35 was observed in broccoli while cabbage and cauliflower did not have harvesting damage. Physiological loss of weight was significantly higher in cauliflower for both packaging types. The net return from broccoli, cabbage and cauliflower packed in plastic crates was higher at Nu.27779.4, Nu.8252.48, Nu.31127.4 compared to Nu.25232, Nu.7540 and Nu.26852 for poly sack packages, respectively. It is recommended that use of plastic crates for handling and transportation of broccoli, cabbage and cauliflower be encouraged and promoted among the stakeholders.

Keywords: *Broccoli; cabbage; cauliflower; transportation damage; PLW; net return*

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1 Introduction

Fresh fruits and vegetables are an important food for the ever-growing population and play a significant role in human nutrition as a main source of vitamins, minerals and dietary fibres (Hailu & Derbew, 2015). In Bhutan, vegetables are cultivated in all parts of the country for self-consumption in the small backyard garden while others cultivate on a large-scale farm for marketing. Total vegetable production in Bhutan was 32,546.07 MT in the year 2021 with broccoli, cabbage and cauliflower production of 1,156.49 MT, 3,763.33 MT and 1,648.18MT, respectively (NSB, 2021). However, there are huge post-harvest losses along the post-harvest chain due to improper handling practices. According to an unpublished report by the National Post Harvest Center, the post-harvest handling losses of cabbage and chilli were reported to be 10.75 % and 8.05 %, respectively in the surveyed areas around Paro and Mongar (Dorji, 2020). A report on the post-harvest losses of vegetables in Bhutan by the UN reported the post-harvest losses of cabbage at 15-20 % and, cauliflower and broccoli at 15-18 % (Acedo Jr & Easdown, 2015).

The post-harvest losses are enhanced due to their perishable nature. Post-harvest losses of fresh produce occur at different handling stages between harvest and consumption along the post-harvest and market value chain. In developing countries, post-harvest losses of vegetables are reported to be as high as 56 % depending on the commodity (Kinhal, 2021). Losses can be largely prevented with timely and accurate harvesting, refrigerated car use in interregional transport, cold storage and the use of packaging material that can prevent moisture loss. Farmers sell their produce in fresh markets or in wholesale markets. At the retail level, fresh produce is sold in an unpackaged form or is tied in bundles. This kind of market handling of fresh produce critically reduces its shelf life if it is not sold quickly (Elik et al., 2019). Packaging is an important factor in reducing losses, as well as extending the shelf life of fresh fruits and vegetables. Therefore, one of the major reasons that fruits and vegetables are lost at post-harvest stages is improper packaging and the use of unsuitable packaging material. Poor quality packaging materials cannot adequately protect fresh produce from damage and can even accelerate the spoilage of fresh produces. Unfortunately, low-quality packaging materials are widely used in many parts of the world due to their low cost. Especially, the use of poor-quality packaging containers is more common in under-developed and developing countries. Even some of delicate fruit and vegetables are packed in poly-sacks that severely damage the delicate products. According to Singh, Hedayetullah, Zaman, and Meher (2014), as much as 40% of the horticultural crops are lost due to high rates of bruising, water loss and subsequent decay which lead to decreased market value of fresh produce mainly due to improper handling, storage, packaging and transportation. As per Dissanayake et al. (2020), post-harvest losses mostly occur due to improper packaging materials and incorrect packaging methods. The use of rigid containers such as plastic crates, wooden boxes and fiberboard boxes minimizes damage occurring in fresh fruits and vegetables during handling and transportation. In Bhutan, post-harvest losses of the vegetable are 16-22 percent for potato, 15-20 percent for cabbage, 22-25 percentage for tomatoes, 20-22 percent for beans, 30-35 percent for peas and 15-18 percent for cauliflower and broccoli (Acedo Jr & Easdown, 2015). The losses are mainly caused due to inadequate handling, packaging, transportation and storage practices resulting in physical damage and spoilage of the produce.

Plastic crates have been widely used in the transportation of fresh vegetables between regions in many parts of the world. In recent years, the use of plastic crates has been encouraged for the handling and transportation of vegetables in Bhutan. While there are some who have adopted the use of plastic crates for the transportation of vegetables, there are others who still prefer sacks to transport the fresh vegetable mainly because of the perceptions that plastic crates are expensive to buy and also requires to be re-transported after the delivery of goods. Plastic crates are stackable, uniform in size and shape and sturdy and are able to prevent damage to the product being handled. Plastic crates are also easy to clean and capable of retaining their full strength while wet. It has the added benefit of reusing for many years (Kitinoja, 2013). Till now there has been no study that compared the economic benefits of using plastic crates over the use of poly sacks in the transportation of vegetables from farms to markets in Bhutan. This study aimed to determine the reduction in post-harvest transportation damage and economic benefit of using plastic crates compared to poly sack for packaging and transportation of broccoli, cabbage and cauliflower from Chali Gewog, Mongar Dzongkhag to the market in Bumthang.

2 Materials and methods

2.1 Harvesting and preparation for market transport

The Cole crop vegetables namely; broccoli, cabbage and cauliflower were harvested and purchased from a farmer’s field in Thempang village, Chali Gewog under Mongar Dzongkhag. A total of 2400 kilograms (800 kilograms of broccoli, 800 kilograms of cabbage and 800 kilograms of cauliflower) of vegetables were purchased and used for the study. Broccoli, cabbage and cauliflower were harvested using a knife in the late afternoon when the weather became cool so as to maintain their freshness for a longer period. The harvested vegetables were moved to a shed area and assessed for harvesting damage (cut, puncture, compression, abrasion, bruising and broken, head damage, etc.). Well-sorted equal quantity of broccoli, cabbage and cauliflower that were free of damage were sorted and packed in plastic crates and poly sack that is most popularly practiced by farmers and vendors in the country. 400 kilograms of broccoli, cabbage and cauliflower were used for each of the packaging types (plastic crates and poly sack). Packaged crates and poly sacks were replicated four times for broccoli and cauliflower while cabbage had three replications. The packed vegetables were transported using a single-cabin bolero vehicle. Broccoli, cabbage and cauliflower were loaded to single cabin bolero and transported to Bumthang town towards the evening on the same day of harvest. The distance from the field in Chali to the Bumthang market is 200 km.

2.2 Physiological loss in weight (PLW)

The vegetables in each replication were weighed before transportation and recorded as the weight of vegetables before transportation. The vegetables were re-weighed individually at the destination market. The physiological loss in weight for vegetables during transportation in the two packaging types was the difference in weight before and after transportation. Weighing of the vegetables was done using a digital weighing scale (WS-10, Bluestar, India). The minimum weighing count of the scale is 50 grams.

2.3 Transportation damage

After the arrival at the destination market in Bumthang town, the heads of broccoli, cabbage and cauliflower were assessed for damages (bruises, cuts, injuries, punctures, etc.,) that must have occurred during the transport in both the packaging type. The number of heads damaged from each replication and packaging type was counted and recorded for analysis. This data was used as the quantity of broccoli, cabbage and cauliflower damaged during transportation from the two packaging types.

2.4 Depreciation cost of plastic crate and cost of poly sack

A technical paper on the use of plastic crates in reducing post-harvest losses and improvement of earnings for fresh produce was referred to calculate the depreciation cost of plastic crates and cost-benefit analysis (Kitinoja, 2013). The depreciation cost of the plastic crate was calculated using the information where the cost of the plastic crate is Nu.650/- with an expected useful life of 3 years. The usage life of a plastic crate in hours is 26,280 hours and the number of hours used per transportation is 24. The cost incurred per usage is Nu.0.59 per use as per the following equation;

$$\text{Cost incurred per usage} = \frac{\text{cost of plastic crate}}{\text{usage life in hours}} * 100$$

The cost of a poly sack is Ngultrum thirty (Nu. 30/-) and this total cost was used in the economic analysis calculation since the sacks were used for a single time.

2.5 Statistical analysis

The data were statistically analyzed using Statistical Package for Social Sciences. Independent sample t-test and Tukey's test was carried out to find the difference in means.

3 Result and discussion

3.1 Between packaging types

The number of heads damaged during transportation was assessed for each of the vegetables and compared between the two packaging types. Transportation damages were significantly higher at $P \leq 0.05$ for samples packed in poly sacks for all the samples compared to the samples from plastic crates (Table 1). Broccoli, cabbage and cauliflower packed in poly sacks reported transportation damage of 8.75±1.5, 8.33±1.5 and 12.5±2.08 numbers of heads, respectively against 0.96±1.1, 0.44±0.09 and 0.41±0.41 numbers of heads for the plastic crate packed vegetable samples. The vegetables packed in plastic crates were properly filled to their capacity and stackable in nature. This ensured that the pressure of weight from vegetable crates did not fall onto each other. This helped in keeping the transportation damage of all three vegetables packed in plastic crates to a minimum. The vegetables packed in poly sacks were stacked upon each other during transportation in the vehicle and the vegetables in the lower part of the vehicle had to take the weight pressure from all the top layers of vegetables packed in the poly sack. As the stacking increased the pressure on the bottom packs increased and this resulted in higher transportation damage for all three types of vegetables packed in poly sacks. The major stretch of the road between Mongar and Bumthang is very rough and unstable and this could have additionally contributed to poly sacks bouncing and rubbing each other thus causing higher transportation damage.

In the post-harvest loss assessment study for apples, it was reported that 9.34 % of apples were partially damaged and 2.70 % damaged totally during the transportation from field to depot. Additionally, 3.69 % of apples were partially damaged while 2.10 % were totally damaged and lost during the transportation from depot to market in Bhutan (Rinchen, Tobgay S., Tshering, & Dorji, 2019). A partial damage of 4.46 % and a complete damage of 1.44 % were reported during the transportation of mandarin fruit in Bhutan from depot to market as per the study by Tobgay et al., 2019. In a transport trial study of eggplant in the Philippines, the compression damage of eggplant packed and transported in polyethylene sack was 54 % while it was reduced to 2.8 % for eggplant packed in plastic crates of 10 kg capacity (Rapusas & Rolle, 2009). The transportation loss of cabbage, carrot and luffa was reduced by 5.8 %, 15.8% and 14.7 %, respectively when plastic crates were used for transportation of these vegetables in Sri Lanka (Wasala et al., 2021). Additionally, the overall visual quality of the vegetables was found to be better retained when transported in plastic crates.

Physiological loss in weight (PLW) per packaging after transportation to the market was assessed from both the packaging type for all three vegetables. Poly-packed samples had significantly higher PLW of 2.02±0.05, 2.83±0.28 kg and 3.4±0.45 kilograms while it was much lower at 0.21±0.01 kg, 0.24±0.01 kg and 0.36±0.02 kilograms for broccoli, cabbage and cauliflower, respectively. The PLW was directly proportional to transportation damage where increased transportation damage resulted in higher PLW. The plastic crate's ability to stack upon each other resulted in lower transportation damage and thus lower PLW. Whereas poly sacks resulted in higher transportation damage that led to increased transpiration from the damaged points and thus increased PLW of the vegetables.

The physiological loss in weight has a direct loss in terms of economic returns since reduced weight means low amount of vegetables to be marketed. The mean physiological weight loss in 2.02 kg of broccoli, 2.83 kg of cabbage and 3.4 kg of cauliflower resulted in monetary losses of Nu.171.7, Nu.99.05 and Nu.323 per sack, respectively. The losses in monetary values for plastic crates samples were much lower at Nu.17.85, Nu.8.4 and Nu.34.2 for broccoli, cabbage and cauliflower at the selling price of Nu.85 for broccoli, Nu.35 for cabbage and Nu.95 for cauliflower.

The lower transportation damage from the plastic crates sample maintained the freshness and attractiveness of the vegetables and vendors were very happy but they were not willing to pay the extra price for it since the customers refuses to buy at an extra price. However, it is recommended that vegetables be transported safely with minimal damage in plastic crates instead of poly sack for premium and long-distance markets.

Table 1. Transportation damage (number of heads) and physiological loss in weight (kg) for broccoli, cabbage and cauliflower between the two-packaging type

Packaging type	Transportation damage (No of heads damaged)			Physiological loss in weight (gm/kg per packaging)		
	Broccoli	Cabbage	Cauliflower	Broccoli	Cabbage	Cauliflower
Poly sack	8.75±1.5a	8.33±1.5a	12.5±2.08a	2.02±0.05a	2.83±0.28a	3.4±0.45a
Plastic crates	0.96±1.1b	0.44±0.09b	0.41±0.41b	0.21±0.01b	0.24±0.01b	0.36±0.02b
P value	0.000	0.001	0.000	0.000	0.004	0.001

Means in the same column with different letters are significantly different between packaging types for each parameter and for each vegetable by independent t-test at $P \leq 0.05$ (Mean ± standard deviation)

3.2 Between vegetables for each packaging type

Harvesting damage was slightly higher for broccoli at 0.35±0.18 number of heads but without any significant difference from cabbage and cauliflower (Table 2). No harvesting damages were reported for cabbage and broccoli. Cabbages and cauliflower were harvested by keeping 3 to 4 outer leaves and this probably protected the heads from getting damaged during harvesting. Harvesting damages seen in broccoli could be due to the injury of side florets that come in the broccoli heads.

For the samples packed in poly packs, transportation damage was significantly higher for cauliflower at 12.5±1.04 heads compared to 8.75±0.75 and 8.33±0.88 heads for broccoli and cabbage. The higher transportation damage of cauliflower in the poly sack could be due to the pressure of weight from each other since cauliflower has more surface area that was exposed and easily got damaged. Transportation damage for the three vegetables was low for plastic crate-packed samples and not significantly different between vegetables as shown in Table 2.

Physiological loss in weight for broccoli was 2.02±0.02 kg and significantly lower compared to the other two vegetables (2.83±0.17 kg for cabbage and 3.40±0.22 for cauliflower) for poly pack samples. Broccoli had the lowest physiological loss in weight of 0.21±0.0 kg even in the plastic crates sample. Cabbage and cauliflower had PLW of 0.24±0.01 kg and 0.36±0.02 kg, respectively and significantly different between the vegetables (Table 2).

Table 2. Harvesting and transportation damage, and physiological loss in weight between vegetables for each packaging type

Vegetable	Harvesting damage	Poly sack		Plastic crates	
		Transportation damage	PLW	Transportation damage	PLW
Broccoli	0.35±0.18[a]	8.75±0.75[b]	2.02±0.02[b]	0.96±0.55[a]	0.21±0.00[c]
Cabbage	0.00±0.00[a]	8.33±0.88[b]	2.83±0.17[a]	0.44±0.06[a]	0.24±0.01[b]
Cauliflower	0.00±0.00[a]	12.5±1.04[a]	3.40±0.22[a]	0.41±0.21[a]	0.36±0.02[a]

Means in the same column with different letters are significantly different between vegetables for each packaging type and for each parameter by Tukey's test at $P \leq 0.05$ (Mean ± standard error)

3.3 Cost-benefit analysis for broccoli between two packaging types

The net return from the sale of 400 kg of broccoli at the market in Bumthang town was estimated at Nu.27,779.4 for plastic crate samples compared to Nu.25,232 for poly sack samples (Table 3 and Figure 1). This translates to broccoli packed in plastic crates earning Nu.2,547.4 more than those packed in poly sacks. The lower physiological loss in weight from the plastic crate sample and the minimal cost of plastic crate per usage (Nu.0.59 per crate per use) when calculated as the depreciation cost over the period of 3 years of expected usage life resulted in fetching higher net return from the sale. The use of plastic crates is recommended for transportation of broccoli to premium and long-distance markets. Cost-benefit analysis of using crates versus woven sacks or traditional baskets was carried out in Rwanda and the researchers found that using plastic crates reduced postharvest losses from 40 % with the woven sacks and 30 % with the traditional sacks to 5 % with the plastic crates. The study also described that plastic crates can be used for several times compared to one or two times for the sacks and the cost of plastic crates would be fully recovered after one use (UC Davis, 2022).

Table 3. Cost-benefit analysis for broccoli between two packaging types

	Plastic crates			Poly sack		
Details	Numbers	Rate (Nu)	Amount (Nu)	Numbers	Rate (Nu)	Amount (Nu)
Packaging type	40	0.59	23.6	16	30	480
Labour	1	500	500	1	500	500
Transportation cost	2 (bolero)		5,000		5,000	5,000
Total cost	5523.6			5,980		
Selling price	391.8*	85	33,303	367.2	85	31,212
Net return**			27,779.4			25,232

*Total weight of broccoli after reaching the market; **Net return is Selling price-Total cost

3.4 Cost-benefit analysis for cabbage between two packaging types

The net return from sales of cabbage packed in plastic crates was Nu.8,252.48 at the market in Bumthang town. The net return from poly sack package was slightly lower at Nu.7,540 for 400 kg of cabbage (Table 4 and Figure 1). The use of returnable plastic crates was studied in Sri Lanka and it was found that the quality and safety of vegetables reaching the consumer were improved significantly (Fernando, 2006). The study also reported that losses for avocados and mangoes were also reduced from 30 % to 6 % when plastic crates were used for handling and transportation.

Table 4. Cost-benefit analysis for cabbage between two packaging types

	Plastic crates			Poly sack		
Details	Numbers	Rate (Nu)	Amount (Nu)	Numbers	Rate (Nu)	Amount (Nu)
Packaging type	28	0.59	16.52	8	30	240
Labour	1	500	500	1	500	500
Transportation cost	2 (bolero)	5,000	5,000		5,000	5,000
Total cost	5,516.52			5,740		
Selling price	393.4*	35	13,769	378	35	13,280
Net return**			8,252.48			7,540

*Total weight of cabbage after reaching the market; **Net return is Selling price-Total cost

3.5 Cost-benefit analysis for cauliflower between two packaging types

The net return from the sale of cauliflower at the destination market was Nu.36,651 from the plastic crate samples. This is Nu.4,275.4 amount more than the net return from poly sack samples that fetched Nu.26,852 (Table 5 and Figure 1). Use of plastic crates for handling, packing and transportation of vegetables is highly recommended since it results in minimal damage and physiological loss in weight and gives high returns. Plastic crates though expensive at first can be used for many years and need not have to procure repeatedly unlike poly sacks which are bought for one-time use. According to one of the reports on returnable plastic crates in post-harvest handling system, users reported that they reuse returnable plastic crates 150 times or more before having to replace them (Kitinoja, 2013). This paper also mentioned that the use of plastic crates can greatly reduce physical damage and reduce fresh fruits and vegetables losses from the typically reported 30 % to 5 % or less.

Table 5. Cost-benefit analysis for cauliflower between two packaging types

	Plastic crates			Poly sack		
Details	Numbers	Rate (Nu)	Amount (Nu)	Numbers	Rate (Nu)	Amount (Nu)
Packaging type	40	0.59	23.6	16	30	480
Labour	1	500	500	1	500	500
Transportation cost	2 (bolero)	5000	5,000		5000	5,000
Total cost	5,523.6			5,980		
Selling price	385.8*	95	36,651	345.6	95	32,832
Net return**			31,127.4			26,852

*Total weight of cauliflower after reaching the market; **Net return is Selling price-Total cost

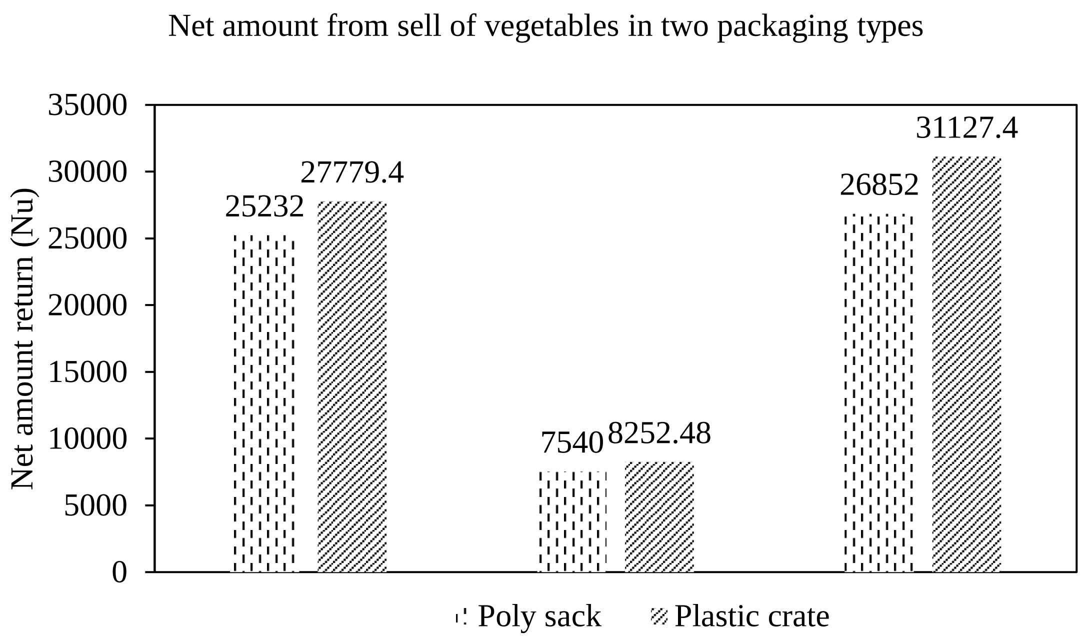

Figure 1. Net return from broccoli, cabbage and cauliflower in the two-packaging type at the market in Bumthang town

4 Conclusion

Packaging and transportation of broccoli, cabbage and cauliflower in plastic crates reduced the transportation damage to vegetables during transportation. Plastic crates maintained the weight of the vegetables with minimum physiological loss. Vegetables packed in poly sacks resulted in higher transportation damage and greater physiological loss of weight that directly translates to a loss of revenue for the stakeholders involved. Broccoli, cabbage and cauliflower packed in plastic crates gave a higher net return compared to those packed in poly sacks. Plastic crates though expensive to buy can be used many times for several years. It can be concluded that the use of plastic crates for packing and transporting can maintain the quality of vegetables through minimized damages, and reduced physiological loss in weight and can thus, enhance the market value. It is recommended that the use of plastic crates be promoted among stakeholders involved in harvesting, handling and transportation of broccoli, cabbage and cauliflower as well as other fresh produces.

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