Detection Survey Protocol for *Acanthoscelides obtectus* Say in Medicinal and Aromatic Plants Nepal

Government of Nepal
Ministry of Agriculture and Livestock Development
Plant Quarantine and Pesticide Management Centre
Hariharbhawan, Lalitpur

March, 2025

Government of Nepal
Ministry of Agriculture and Livestock Development
Plant Quarantine and Pesticide Management Centre
Hariharbhawan, Lalitpur

Detection Survey Protocol for *Acanthoscelides obtectus* Say in Nepal

NPPO-Nepal, 2025

Table of Contents

SN	Title	Page
1	Introduction	1
2	Taxonomic tree	2
3	Biology/life cycle	2
4	Field identification	3
5	Mode of dispersal	4
6	Host range	4
7	Detection survey	5
	7.1 Visual inspection method	5
	7.2. Trapping method	5
8	Habitat	5
9	Reports in Nepal	5
10	Purpose	6
11	Scope	6
12	Target pest	6
13	Timing of survey	6
14	Location of survey	6
15	Design of survey program	7
	15.1 Sampling method	7
	15.2 Sample size	7
16	Materials required	8
17	Collection and preservation of specimen	9
	17.1. Collection from storage	9
	17.2. Collection from field	9
	17.3. Preservation of specimen	10
	17.4. Labeling the specimen	10
18	Morphological diagnosis	10
19	Molecular diagnosis	11
20	Sample analysis and reporting	11
21	Literature cited	11
	Annexes	13
	Annex-1 Calendar of activities to follow while conducting detection survey	13
	Annex-2 Location-wise monitoring and observation	13
	Annex-3 Details of observation to be recorded in the storage while surveying	14
	Annex-4 Data to be recorded during the survey in field	14
	Annex-5 Data to be recorded during the survey in store	14

Government of Nepal
Ministry of Agriculture and Livestock Development
Plant Quarantine and Pesticide Management Center
Hariharbhawan, Lalitpur

3/31/2025

Detection Survey Protocol for *Acanthoscelides obtectus* Say in Nepal

NPPO-Nepal, 2025

Endorsed by NPPO-Nepal on March 3, 2025

1. Introduction

Acanthoscelides obtectus (Say, 1831) is a serious pest of stored common bean (*Phaseolus vulgaris* L.). In common beans, it can cause 40% loss if left without treating in the storage (Savkovic et al., 2019). First, this pest attacks the host in the field and later, it gets into storage areas (Alvarez et al., 2005). Apart from common bean, it also attacks other host species belonging to fabaceae family. The host range of *A. obtectus* has been expanding since 1960s and may continue to expand (Alvarez et al., 2005). It is a cosmopolitan insect and has been reported from all the continents excluding Antartica (Hervet et al., 2023). There is a conflict among scientists regarding its origin. Some reported its origin to be Neotropical (Alvarez et al., 2005), while some considers it to be around Central America (Savkovic et al., 2019). It is found in tropical, subtropical and temperate zones of the world (Baier & Webster, 1992; Alvarez et al., 2005). It is relatively more tolerant towards cold temperatures (Hervet et al., 2023).

China has listed *A. obtectus* as a quarantine pest while exporting medicinal plants from Nepal. The agreement signed between General Administration of Customs of the People's Republic of China (GACC) and Government of Nepal (GoN) has provision of ensuring the medicinal plants to be exported from Nepal should be free from this pest. Plant Quarantine and Pesticide Management Centre is authorized by the government of Nepal as NPPO, and under Plant Protection Act 2064, Clause 6 (2), survey and surveillance function and responsibility is designated to NPPO as per the sub clause (i) "To perform such other function as prescribed"

For the continuous and effective trade of medicinal plants between Nepal and China, the detection survey for the presence of this pest has to be carried in regions where the plants are being produced, stored or processed while, it could be of serious concern in already infested storage where the medicinal plant products are being stored. Because, proper pest detection and pest identification are crucial for the appropriate application of phytosanitary measures (ISPM-4 (Requirements for the establishment of pest free areas), ISPM-6 (Guidelines for surveillance), ISPM-7 (Phytosanitary certification system), ISPM-9 (Guidelines for pest eradication programs) and ISPM- 20 (Guidelines for a phytosanitary import regulatory system) (FAO, 2020). This survey protocol may guide the surveyors, government officials in the quarantine check-post and others for conducting the detection survey and identifying the pest successfully.

3/3/2025

Government of Nepal
Ministry of Agriculture and Livestock Development
Plant Quarantine and Pesticide Management Center
Hariharbhawan, Lalitpur

3/3/2025

2. Taxonomic tree

Domain: Eukaryota
Kingdom: Animalia
Phylum: Arthropoda
Subphylum: Hexapoda
Class: Insecta
Order: Coleoptera
Family: Chrysomelidae
Sub- family: Bruchinae
Genus: *Acanthoscelides*
Species: *Acanthoscelides obtectus* Say

Source: (EPPO, 2002)

3. Biology/life cycle

A. obtectus is a multivoltine species and has high reproductive potential under various ecological domains (Alvarez et al., 2005).They are widely adaptive and tolerant to cold (Hervet et al., 2023) as well as hot temperature ranges (CABI, 2022). But the reproduction is slow below 18°C. Most favorable conditions for development of this pest are around 30°C and 70% RH (CABI, 2022). The minimum temperature for the completion of life cycle is 11°C and maximum temperature is 36°C (Soares et al., 2015). The life cycle is completed in minimum 21 to maximum 80 days depending upon the temperature, relative humidity and host plant (University of Florida, 2005). At 30°C and 70% RH, it took about 28 days, and at 23°C and 80% RH, 50-60 days to complete one complete cycle (Baier & Webster, 1992).

Commonly, the adult does not feed and survives on the stored energy during larval feeding and development. After two hour of adult emergence, they are ready to mate and reproduce (Savkovic et al., 2019) The adult female lays an average of 70-75 eggs singly or in clusters on the surface of host plants (beans) (University of Florida, 2005). Unlike other bruchids, they lay eggs loosely on the cracked host or making holes on host seeds (Hervet et al., 2023). Since, they lay eggs loosely, they can easily get transferred to other host as well in the egg stage (Savkovic et al., 2019). There are four larval stages. The first instar larvae search for the hosts, bore and feed inside them (University of Florida, 2005). Remaining larval instars feed inside the endosperm and remains there until it becomes pupa and emerges out as an adult (Hervet et al., 2023). The larva and pupal stages lasts around 30 days under optimum conditions. As they emerge as an adult, leaves windows or small holes (University of Florida, 2005).

3/31/2025

Government of Nepal
Ministry of Agriculture and Livestock Development
Plant Quarantine and Pesticide Management Center
Hariharbhawan, Lalitpur

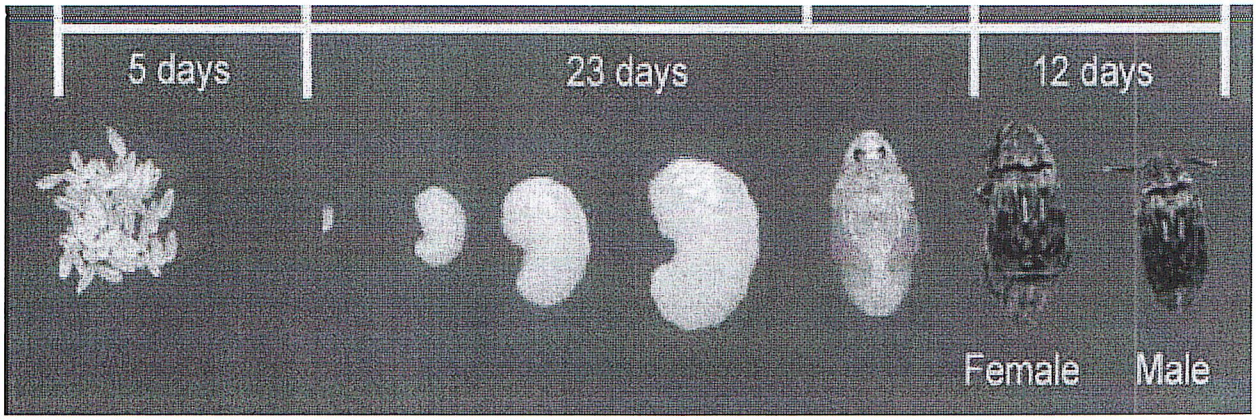


Figure 1. Life cycle of *A. obtectus*

Source: (CABI, 2022)

4. Field identification

Adult: Olive brown adults mottled with dark-brown and gray (Without any significant pattern) are 2 to 3 mm long (University of Florida, 2005). The body length is approximately double the width of the specimen. The female adult have larger body size than male adults (Thakur, 2012). The hind femur of adult beetle possesses one large and two small spines as shown in figure 3 (University of Florida, 2005). The larger spine is almost two times longer than small spines. The two small spines are equal in length (Thakur, 2012). The abdomen (ventral half of the hind femur is black) and legs are reddish in color (University of Florida, 2005). They have 11 segmented antennae in which first to fourth segments are filiform, fifth to tenth segments are slightly widened and serrated and the last segment is non serrated with acute apex (Thakur, 2012). The antenna is dark grey except (1-5) segments, which are reddish colored (CABI, 2022). The setae (prothorax and elytra) are majorly yellowish-grey with darker brown patches. The pygidium of male is vertical and only partly visible from above but due to oblique nature, it is sub-vertical and easily visible in females from above (CABI, 2022). The male pygidium (Color and shape) is very crucial for distinguishing it from other species of *Acanthoscelides*. The color of pygidium is orange. The lateral lobe present on the male aedeagus is smooth and thin unlike other species (Alvarez et al., 2004). Their feeding damage clearly show hole on stored beans.

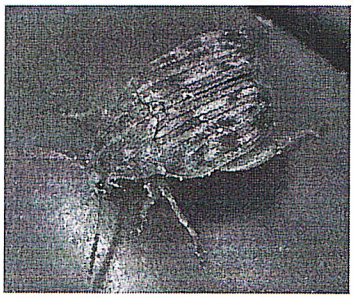


Fig. 3: Adult of *A. obtectus*

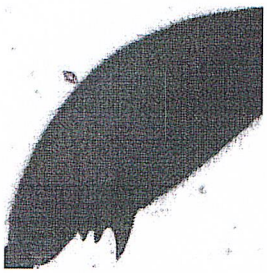


Fig. 4: Femur of adult *A. obtectus*

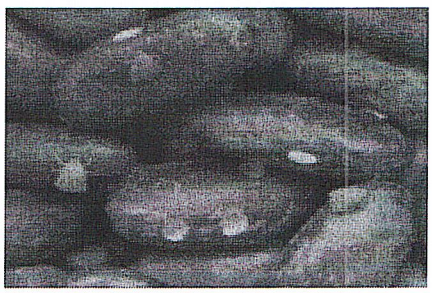


Fig. 5: Damage symptom of *A. obtectus* in common bean
Source: (Thakur, 2012)

3/31/2025

Government of Nepal
Ministry of Agriculture and Livestock Development
Plant Quarantine and Pesticide Management Center
Hariharbhawan, Lalitpur

5. Mode of dispersal

The adult can fly short distances (Rodriguez, 2018) and can crawl for dispersing in short distance. However, it has been mostly dispersed and migrated with humans (Alvarez et al., 2005). The pest may travel long distances through trade of infested grains and medical products across the countries or regions.

6. Host range

A. obtectus have narrow host range and oligophagous in nature (Alvarez et al., 2004). It is present in domestic as well as wild hosts (Baier & Webster, 1992). Some of the host as described by CABI (2022) are as follows:

- Main hosts: Common bean (*Phaseolus vulgaris*), lima bean (*Phaseolus lunatus*)
- Other hosts: Chickpea (*Cicer arietinum*) and cowpea (*Vigna unguiculata*)
- Wild host: Runner bean (*Phaseolus coccineus*)

However, the concerned medicinal plants have not been detected as host of *A. obtectus* yet. *A. obtectus* is the quarantine pest of following hosts.

S N	Scientific Name	S N	Scientific Name
1	*Phyllanthus emblica*	9	*Polygonatum kingianum* *Polygonatum sibiricum* *Polygonatum cyrtonema* *Polygonatum cirrhifolium* *Polygonatum verticillatum*
2	*Paris polyphylla*	10	*Amomum subulatum*
3	*Aquilaria sinensis* *Aquilaria malaccensis* *Aquilaria agallocha*	11	*Ganoderma lucidum* *Ganoderma sinense*
4	*Herpetospermum pedunculosum*	12	*Rubia wallichiana* *Rubia tibetica* *Rubia spp.*
5	*Murraya exotica* *Murraya paniculata* *Murraya koenigii*	13	*Piper longum*
6	*Cassia obtusifolia* *Cassia tora* *Senna tora*	14	*Ferula sinkiangensis* *Ferula fukanensis* *Ferula narthrex*
7	*Santalum album*	15	*Justicia adhatoda*
8	*Swertia chirayita*		

3/31/2025

Government of Nepal
Ministry of Agriculture and Livestock Development
Plant Quarantine and Pesticide Management Center
Hariharbhawan, Lalitpur

7. Detection survey

Before doing detection, the General Surveillance is done to know whether the insect is present in the country or not. In case of its presence, the detection survey is conducted with the methods described below:

7.1 Visual inspection method

In the visual inspection method, the warehouse, equipment used in the storage, bags, surroundings, cracks and crevices around the storage structure should be carefully inspected. Careful search, observation and accurate recording of results must be practiced following reliable method. The method relies on the knowledge and experience of the surveyor. During carrying out inspection, some of the randomly chosen bags should be sampled, as well as bags corner, surface, folds should be examined. Some of the bags should be lifted and agitated to trace the infestations (Semple et al., 1992). Some of the hosts might have kept closer to the medicinal plants that might be the cause of infestation. In such case, the infestation in the nearby storage of kidney bean or common bean or any other host plants should be examined. The packaging and processing areas of medicinal plants should also be included for inspection.

7.2 Trapping method

Traps offer a potential approach for estimating the degree of infestation and can also be used to detect light infestations (Semple et al., 1992). There are different types of traps that can be used for the detection of *A. obtectus,* such as probe trap, pitfall trap, pheromone trap, phytochemical based attraction lure, etc. Appropriate trap can be set depending on the situation i.e. in the field and stored conditions. For example, Probe type trap is a method that can be used in storage bags to determine the presence of the pest. In this method, the probe is inserted inside the store bags and left for three hours. The *A. obtectus* adult crawling inside the bag can be collected with this method. The pitfall trap is used in the field for monitoring the pest using general type of pitfall trap. Depending on the availability on the market, combination of six male pheromonal compounds (methyl (E,R)-2,4,5-tetradecatrienoate, methyl (2E,4Z,7Z)- 2,4,7-decatrienoate, methyl (2E,4Z)-2,4-decadienoate, octadecanal and the sesquiterpenes (3Z,6E)- and (3E,6E)-α-farn) are successful in attracting the female adults (Vuts et al., 2015). The pest is also a pollen and nectar generic feeder and one of the chemical present inside the pollen that attracts *A. obtectus* towards pollen and nectar is benzyl alcohol and methyl anthranilate. When benzyl alcohol is used individually or in combination with methyl anthranilate as attraction lure, it can trap large number of adults (Vuts et al., 2021). This trap can be useful in attracting the beetles in field as well as in storage.

8. Habitat

A. obtectus can survive in wide range of temperature regimes though its growth and development is somewhat slower in less than 18°C. It survives from cool highland areas of the tropics to warmer lowland tropics, also in temperate regions (CABI, 2022).

9. Reports in Nepal

It has not yet been reported in Nepal. But, it has been reported in neighboring country India (from mid-Himalayan region in 2012) (Thakur, 2012). Given the continuous trade of Nepal with India, there are chances of entering in Nepal along with import products, with possibility of finding its presence in the detection survey.

3/13/2025

Government of Nepal
Ministry of Agriculture and Livestock Development
Plant Quarantine and Pesticide Management Center
Hariharbhawan, Lalitpur

10. Purpose

- To detect *Acanthoscelides obtectus* Say from the randomly selected medicinal plants in production areas (sample to be taken in areas from where the medicinal plant products are collected).
- To support NPPO to declare pest free area.
- To report to organization such as IPPC, GACC etc. for the facilitation of trade of medicinal plants.

11. Scope

The survey will cover the randomly selected storage and field locations. The host location specific details will be collected from various sources like Department of Plant Resources, Department of Forest and soil conservation, National Herbarium and Plant Laboratories, and other related institutions like NARC Research Stations, Central Department of Botany of Tribhuvan University, Kathmandu University, Agriculture and Forestry University, NGO, INGOs and other published materials.

12. Target pest

- **Preferred scientific name**: *Acanthoscelides obtectus* Say
- **English common name**: American seed beetle, bean beetle, bean weevil, common bean weevil, dried bean beetle, dried bean weevil.

13. Timing of survey

Time of survey or sampling schedule of *A. obtectus* is given in Table 1. The pest is generally active throughout the year and does not undergo any diapause stage (Rodriguez, 2018). But, the optimum temperature for the growth and reproduction is around 20-28°C (Soares et al., 2015). The suitable time in Nepal is in between May- September for peak infestation. It can also be surveyed other time of the year except the time when the temperature is below 11°C and above 34°C.

Table 1. Sampling schedule for detection survey of *A. obtectus* across various sites

Production site	Sampling frequency			
	June	July	August	September
Site 1	June (1st week)	July (1st week)	August (1st week)	September (1st week)
Site 2	June (2nd week)	July (2nd week)	August (2nd week)	September (2nd week)
Site 3	June (3rd week)	July (3rd week)	August (3rd week)	September (3rd week)
Site 4	June (4th week)	July (4th week)	August (4th week)	September (4th week)

14. Location of survey

Survey will be conducted in local storage house, warehouses, packaging and processing areas of medicinal and aromatic plants products. The field (wild or cultivated), where the medicinal plants are produced or collected will also be included in the survey.

Government of Nepal
Ministry of Agriculture and Livestock Development
Plant Quarantine and Pesticide Management Center
Hariharbhawan, Lalitpur

15. Design of survey program

15.1 Sampling method

A. obtectus is the common storage pest and mostly found in the storage areas. For the pest detection, storage area should be checked carefully, i.e. entry, exit and surrounding areas. The possible pathway of occurrence of this pest in the storage should be observed for example: the movement of products, containers, or people handling such products, which might get exposed to this beetle should be checked. Storage structures such as local storage equipment, sacks, woodwork, loose plaster, loose paint, cracks and other potential hiding spots should be observed. The proximity of nearby bean storage should also be observed (NPPO, 2022).

For sampling in storage area, random number should be assigned to the sampling units such as the stored bags with the random sampling applied for selection and inspection for the detection of the pest. While surveying in the field, adequate, representative samples should be collected that will support in accurately detecting the pest. Employing single approach is not effective for conducting surveys in the field (Saikia, 2023). Diagonal methods are used for quickly scanning the presence of pest. However, more representative samples are obtained from W diagonal and random sampling method. For detection survey, W method or random sampling design can be used.

15.2 Sample size

Total sample size will be calculated based on the following formula:

$$\text{Sample size} = \frac{-\log(1 - \text{confidence level})}{\log(1 - \text{Design prevalence}}$$

The design prevalence for detection survey is 1% (NPPO-Nepal, 2024). Hence, from the Table 2, minimum 298 samples should be taken for detecting the pest at 95% confidence level.

Table 2. Calculated sample size for different design prevalence at different confidence level

Confidence Level	1 – Confidence Level	Design Prevalence	1 – Design Prevalence	Sample Size
0.95	0.05	0.01	0.99	298
0.95	0.05	0.02	0.98	148
0.99	0.01	0.01	0.99	458
0.99	0.01	0.02	0.98	228
0.95	0.05	0.001	0.999	2,994
0.95	0.05	0.002	0.998	1,496
0.99	0.01	0.001	0.999	4,603
0.99	0.01	0.002	0.998	2,300

Number of samples per sampling point differs according to the medicinal plant species to be observed. Table 3 gives the criteria for selecting the minimum number of samples per sampling point.

3/13/2025

Government of Nepal
Ministry of Agriculture and Livestock Development
Plant Quarantine and Pesticide Management Center
Hariharbhawan, Lalitpur

Table 3. Minimum number of samples to be obtained from each sampling point

Medicinal plants	Minimum number of samples
Herbs species *(Swertia chirayita, Polygonatum* spp*)*	5 plants/ sampling point
Specimen associated with tree *(Ganoderma lucidum)*	2 tree per sampling point and 2 mushroom/ tree
Tree species (*Phyllantha emblica, Piper longum)*	2 tree/ sampling point and 10 fruits per tree

16. Materials required

The listed equipment are essential for conducting survey in storage area.

- Containers with ventilation (for live insect)
- Collection jar
- Magnifying lens
- Camel hair brush
- Ziplock bag
- Data-sheet
- Magnifying lens
- Diagnostic keys
- Envelops
- Alcohol/water resistant pen
- Labels
- Killing jar
- Absolute alcohol
- GPS measuring tool
- Camera/ mobile phone
- Markers
- Tweezers
- Traps/lures
- Collector tags
- Cotton rolls
- Ethyl acetate
- Glass vials
- Data sheets
- Zip-lock bag

The listed equipment are needed for the field survey.

- Mosquito repellent
- GPS measuring tool or Geometer
- First-aid kit
- Permits
- Camera or mobile phone
- Data-sheets

3/13/2025

Government of Nepal
Ministry of Agriculture and Livestock Development
Plant Quarantine and Pesticide Management Center
Hariharbhawan, Lalitpur

- Field guide
- Water-proof/alcohol-proof pens
- Labels
- Zip-lock bag
- Paper bags
- Note-book
- Magnifying lens
- Insect collection tubes
- Absolute alcohol and 70-90% alcohol
- Forceps
- Ethyl acetate
- Cotton rolls
- Identification keys of the specimen
- Measuring tape
- Aspirator
- Traps

Source: (NPPO-Nepal, 2024)

17. Collection and preservation of specimen

17.1 Collection from storage

- Adult, larva and pupal stages of insects should be collected with sterilized camel hair brush or by using an aspirator.
- Specimens should be placed in well ventilated container for safe shipping if the identification is not done immediately at the same locality.
- For convenience of handling, the adult in the container can be kept in the freezing temperature for 2 hours (NPPO-Nepal, 2024).
- For collecting larva and pupa, the seed sample should also be collected. If found sample seeds with visible eggs on the surface of the seed, such samples should be collected and handled carefully to avoid the damage to the eggs.
- Multiple samples are always better.

17.2 Collection from field

According to NPPO-Nepal (2024), following things need to be considered while collecting the specimen from the field.

- Before collecting, the equipment needed for collection should be sterilized with 70% ethanol or 0.5% chlorine solution.
- The specimen should be collected and handled very carefully as it may damage its diagnostic feature for identification due to carelessness.
- All the life stages of specimen should be collected whenever possible.
- The collected specimens must have intact appendages like antennae, wings, and legs.
- The adult weevil should be folded in tissue paper to protect its appendages. Well ventilated container is most suitable for transportation.
- If possible the sample should be stored in a secure, cool, and dark place. The stored sample can be kept in a freezer for 2 hours before dispatch to kill the insect for the convenience of handling.

3/13/2025

Government of Nepal
Ministry of Agriculture and Livestock Development
Plant Quarantine and Pesticide Management Center
Hariharbhawan, Lalitpur

- All the samples should be well-labelled.

17.3 Preservation of specimen

The most common technique for preserving immature insects is to collect them and store them in a vial filled with 70% ethanol (alcohol). Boiling in water before preservation can be used to maintain color in soft bodied insects and then placed in 65% ethyl alcohol and the container should be completely filled to prevent its movement and damage (NPPO-Nepal, 2024). Immature stages are preserved in fluid (stored in 85–90% ethanol, preferably after fixation in KAA or Carnoy's fluid).

17.4 Labeling the specimen

The collected weevil should be labelled with the help of alcohol or water resistant inks. The labelling should be done both inside and outside the jar. While labelling the specimen following this need to be considered in the label (NPPO-Nepal, 2024):

- Host name (Scientific and common name)
- Host commodity Plant parts affected by the pest
- Pest's scientific name and life stage
- Family or order of the pest
- Location details
- Collection date
- Name of collector

18. Morphological diagnosis

For successful identification of *A. obtectus*, adult specimen is used. The shape and color of adult antennae, femur, male and female genitalia should be observed (Alvarez et al., 2005). The method of preparation of adult for identification of weevil as described by Seram et al. (2022) is described below.

I. Well preserved specimen with all the appendages and organs should be selected for dissection and identification of the species.
II. The selected specimen and equipment should be well sterilized in 70% ethanol or 0.1% Sodium hypochlorite solution for one minute.
III. The sterilized adults should be kept in separate vials with 10% potassium hydroxide solution. This helps in softening of tissues and dissolution of fat.
IV. The abdominal parts containing the genital organs should be dissected which can be done with the help of stereomicroscope.
V. The aedeagus of male weevil should be carefully dissected. For this operation, microneedle and forceps can be used. Special consideration should be given while carrying out this step to avoid damage to the organs.
VI. The aedeagus should be cleaned and stained with the help of staining agent (Fuchsin acid dissolved in acetic acid).
VII. For improved transparency, the stained parts should be transferred to the clearing solution (2:3 carboxylic acid + xylene solution).
VIII. Now, the specimen should be mounted on the observing glass slides using Canada balsam. Glycerol can also be used as a mounting agent for preparing temporary slide.

Government of Nepal
Ministry of Agriculture and Livestock Development
Plant Quarantine and Pesticide Management Center
Hariharbhawan, Lalitpur

IX. The female genitalia (8th and 9th segment), legs, forewings, antennae should also be prepared in a permanent or temporary slide following the step I to step VIII.

19. Molecular diagnosis

Molecular diagnosis of *A. obtectus* can be done by several methods such as real time Polymerase Chain Reaction (RT-qPCR), DNA barcoding and DNA sequencing. For carrying out PCR for diagnosis of *A. obtectus*, the universal primer can be used (Roy et al., 2023). For the molecular diagnosis, the well-preserved specimen are sent to the molecular laboratories of Nepal Agriculture Research Council (NARC), Central Agriculture Laboratory, Universities (AFU, TU) and other accredited private laboratories.

20. Sample analysis and reporting

Surveyor should keep at least one specimen with himself and at least one specimen should be sent to NPPO for identification and future reference. The laboratory should send the report to the NPPO if it has examined and identified the specimen. They should notify the NPPO for the reporting/declaration of insect-pest if the specimen is examined and identified by the Central Agricultural Laboratory, the National Entomology Research Center, or any other organization. The specimen shall be preserved and all records should be securely stored by NPPO (NPPO-Nepal, 2024).

21. Literature cited

Alvarez, N., McKey, D., Hossaert-McKey, M., Born, C., Mercier, L., & Benrey, B. (2004). Ancient and recent evolutionary history of the bruchid beetle, *Acanthoscelides obtectus* Say, a cosmopolitan pest of beans. *Molecular Ecology, 14*(4), 1015–1024.

Alvarez, N., Hossaert-McKey, M., Rasplus, J.Y., McKey, D., Mercier, L., Soldati, L., Aebi, A., Shani, T., & Benrey, B. (2005). Sibling species of bean bruchids: A morphological and phylogenetic study of *Acanthoscelides obtectus* Say and *Acanthoscelides obvelatus* Bridwell. *Journal of Zoological Systematics and Evolutionary Research, 43*(1), 29–37.

Baier, A.H., & Webster, B.D. (1992). Control of *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae) in *Phaseolus vulgaris* L. seed stored on small farms-I. Evaluation of damage. *Journal of Stored Products Research, 28*(4), 289–293.

CABI Compendium. (2022). *Acanthoscelides obtectus* (bean bruchid). *CABI International*. Retrieved from: https://www.cabi.org

EPPO. (2002). *Acanthoscelides obtectus*. *EPPO Global Database*. Retrieved from: https://gd.eppo.int/taxon/ACANOB

FAO. (2020). International year of plant health. https://www.fao.org/plant-health-2020/about/en/| Food and Agriculture Organization of the United Nations.

Hervet, V.A., Fields, P.G., Hamilton, K.D., Nadimi, M., & Paliwal, J. (2023). Cold tolerance of *Acanthoscelides obtectus*, the bean weevil: Effects of cold acclimation, life stage and strain. *Journal of Stored Products Research, 104*, 102-169.

NPPO-Nepal. (2024). Technical guidelines for surveillance of plant pests in Nepal. PQPMC, Hariharbhawan, Lalitpur, Nepal.

Rodriguez, S.A. (2018). Semiochemistry of the Bruchidae species. *Journal of Stored Products Research, 77*, 148–155.

3/13/2025

Government of Nepal
Ministry of Agriculture and Livestock Development
Plant Quarantine and Pesticide Management Center
Hariharbhawan, Lalitpur

Roy, V., Mpika, J., Kergoat, G. J., Mboussy, G.F.T., & Attibayeba, A. (2023). Diversity of insect pests of common bean and pigeon pea in the Republic of Congo revealed by DNA barcoding. *African Entomology, 31*, e15329.

Saikia, J. (2023). *Pest survey and surveillance* [PowerPoint slides]. SlideShare. Retrieved from https://www.slideshare.net/slideshow/pest-survey-and-surveillancepptx/259254713

Savkovic, U., Djordjevic, M., & Stojkovic, B. (2019). Potential for *Acanthoscelides obtectus* to adapt to new hosts seen in laboratory selection experiments. *Insects, 10*(6), 153.

Semple, R.L., Hicks, P.A., Lozare, J.V., & Castermans, A. (Eds.). (1992). Towards integrated commodity and pest management in grain storage: A training manual for application in humid tropical storage systems. *Proceedings and Selected Papers from the Regional Training Course on Integrated Pest Management Strategies in Grain Storage Systems, National Post Harvest Institute for Research and Extension (NAPHIRE)*, Department of Agriculture, Philippines, June 6–18, 1988.

Seram, D., Natesan, S., Pandiyan, M., Karthikeyan, A., & Kennedy, J. (2022). Differentiating *Callosobruchus* of South India with special reference to *Callosobruchus maculatus* - A useful guide for entomologists and non-entomologists. *Preprints*. https://doi.org/10.21203/rs.3.rs-1783177/v1

Soares, M.A., Quintela, E.D., Mascarin, G.M., & Arthurs, S.P. (2015). Effect of temperature on the development and feeding behavior of *Acanthoscelides obtectus* (Chrysomelidae: Bruchinae) on dry bean (*Phaseolus vulgaris* L.). *Journal of Stored Products Research, 61*, 90–96.

Thakur, D.R. (2012). Taxonomy, distribution, and pest status of Indian biotypes of *Acanthoscelides obtectus* (Coleoptera: Chrysomelidae: Bruchinae) - A new record. *Pakistan Journal of Zoology, 44*(1), 189–195.

University of Florida. (2005). Bean weevil. Retrieved from: https://entnemdept.ufl.edu/projex/gallery/dl/Stored_Product_Pests/text/bean_weevil.htm

Vuts, J., Powers, S. J., Caulfield, J. C., Pickett, J. A., & Birkett, M. A. (2015). Multiple roles of a male-specific compound in the sexual behavior of the dried bean beetle, *Acanthoscelides obtectus*. *Journal of Chemical Ecology, 41*, 287–293.

Vuts, J., Szanyi, S., Szanyi, K., König, L., Nagy, A., Imrei, Z., & Tóth, M. (2021). Development of a phytochemical-based lure for the dried bean beetle *Acanthoscelides obtectus* Say (Coleoptera: Chrysomelidae). *Journal of Chemical Ecology, 47*(12), 987–997.

3/13/2025

Government of Nepal
Ministry of Agriculture and Livestock Development
Plant Quarantine and Pesticide Management Center
Hariharbhawan, Lalitpur

Annexes

Annex 1. Calendar of activities to follow while conducting detection survey

Phase	Time of the year	Activities
Pre-survey preparations	February-March	Literature review Thoroughly understanding the protocol Training of the surveyors Purchasing the materials required for survey. Co-ordination with the traders, farmers/collectors. Budget allocation for survey
Field survey	May	1st replication of survey
	June	2nd replication of survey
	July	3rd replication of survey
	August	4th replication of survey
Laboratory diagnostics	September-October	Submitting the samples to laboratory for morphological and molecular analysis
Analysis/ reporting	November-December	Preparation of Survey report Submission to NPPO-Nepal Conducting Validation workshop.

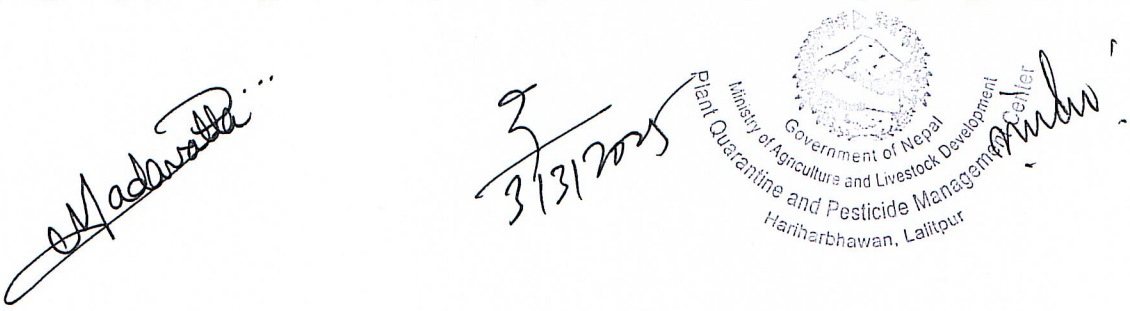
Annex 2. Location-wise monitoring and observation

Medicinal plant producing districts where there is potential of *A. obtectus* detection are listed below:

Region	**Districts**
Mountain	Solukhumbu, Taplejung, Mustang, Mugu, Dolpa
Hill	Dhankuta, Ilam, Panchthar, Terhathum, Sankhuwasabha, Okhaldhunga, Bhojpur, Khotang, Udaypur, Dolakha, Ramechhap, Sindhupalchok, Dhading, Makwanpur, Tanahun, Syangja, Gorkha, Lamjung, Palpa, Gulmi, Myagdi, Baglung, Rukum, Dailekh, Dadeldhura, Bajhang

Proposed districts to be carrying out detection survey of *A. obtectus* in 2081/82

Taplejung, Mugu, Khotang, Terhathum, Sindhupalchok, Tanahu, Gorkha, Bajhang



Annex 3. Details to be recorded in the storage while surveying

Title of the study/survey	
Name of field/site visited	
Date/time of field visit when the pest was intercepted	
Name and the address of local people involved	
Contact details of local people/s involved in the survey	Phone: Email:
GPS reference point	Latitude:
	Longitude:
	Altitude:
Locality	Village name ward no.:
	Local level:
	District:
Climate data of locality	Average min. temp (in °C):
	Average max. temp (in °C):
	Rainfall (in mm)

Annex 4. Data to be recorded during the survey in field

Date: Host:

Sample number	Infestation Level (Low, medium and high)	No. of Insects Observed	Damage Symptoms	Stage of insect observed	Remarks

Annex 5. Data to be recorded while carrying out survey in storage

Date:

Host commodity:

Trap type	Trap density	Location of trap	Insect captured/ trap	Life stage captured	Remarks

3/13/2025

Government of Nepal
Ministry of Agriculture and Livestock Development
Plant Quarantine and Pesticide Management Center
Hariharbhawan, Lalitpur