Detection Survey Protocol for *Callosobruchus analis* Fabricius 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 *Callosobruchus analis* Fabricius in Nepal

NPPO-Nepal, 2025

Table of Contents

3/13/2025

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

Detection Survey Protocol for *Callosobruchus analis* Fabricius in Nepal
NPPO-Nepal, 2025

Endorsed by NPPO-Nepal on March 3, 2025

1. Introduction

Callosobruchus analis (Fabricius, 1781) is a significant stored pest of grain legumes. Among different grain legume, cowpea (*Vigna unguiculata*) and green gram (*Vigna radiata*) are the preferred host for higher oviposition, growth and development under normal storage conditions (Sarwar et al., 2012). It severely affects the quality of infested grain legumes for example, protein quality of gram. Also cause reduction in energy, starch, total sugar and non-reducing sugars level. It can cause damage up to 38-62% in various chickpea genotypes (Sarwar et al., 2012).

China has listed *A. analis* 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.

2. Taxonomic tree

Domain: Eukaryota
Kingdom: Animalia
Phylum: Arthropoda
Subphylum: Hexapoda
Class: Insecta
Order: Coleoptera
Family: Bruchidae
Genus: *Callosobruchus*
Species: *Callosobruchus analis* (Fabricius, 1781)

Source: (CABI, 2019)

3/3/2025

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

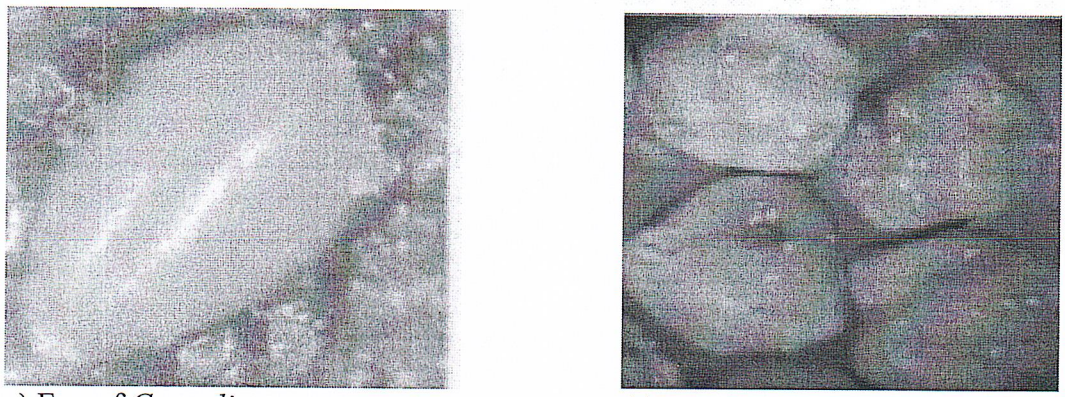
3/3/2025

3. Biology/life cycle

C. analis undergoes complete metamorphosis with distinct egg, larva, pupa and adult stages. The life cycle completes within two months under optimal conditions but may take longer period when the temperature and relative humidity is below the optimal conditions. Under conditions of average temperature of 28.7 °C and 75.2 % RH, the life cycle is reported to complete in 53–62 days (Devi & Devi, 2014). Development slows significantly below 18°C or above 35°C with the cycle extending to approximately 94 days at 20°C and 70% RH (CABI, 2019). *C. analis* life cycle and biology also depends on the host species (Mannava et al., 2022).

Female *C. analis* can store sperm throughout their lifetime and mate with multiple males. The mating completes within an hour of emergence (CABI, 2019). Female adult lays about 200 eggs singly on the host seed testa. For breeding and egg laying, the optimal conditions for *C. analis* is 30°–35°C and 70% RH (CABI, 2019). The average duration when the egg hatches is 8-9 days (Devi & Devi, 2014). Upon hatching, the larvae chews egg's base, penetrate inside the seed coat, and burrows inside the seed. The debris generated during this process is attached to the hatched egg, making it easily visible. The larvae develops entirely within a seed. There are four larval stages where, first, second, third and fourth instar lasts 10-11 days, 5-6 days, 5-6 days and 4-5 days respectively (Devi & Devi, 2014). The average larval development period is about 27 days. The larva undergoes into pre-pupal stages and then into pupal stages. The pupal stage lasts 5-7 days. The female adult emerges later than the male adult (Devi & Devi, 2014). Adults do not feed. Energy reserves accumulated during the larval stage is used in adult stage for survival and reproduction (CABI, 2019).

Eggs: Eggs are oval shaped, tapering at one end. The length of egg is 650 µm and 400 µm wide at the widest point. Freshly laid eggs by female adults are translucent and inconspicuous. However, the fertile eggs are white or milky opaque (CABI, 2019).



a) Egg of *C. analis* b) Egg laid on the host's surface

Fig. 1: Egg stage of *C. analis*

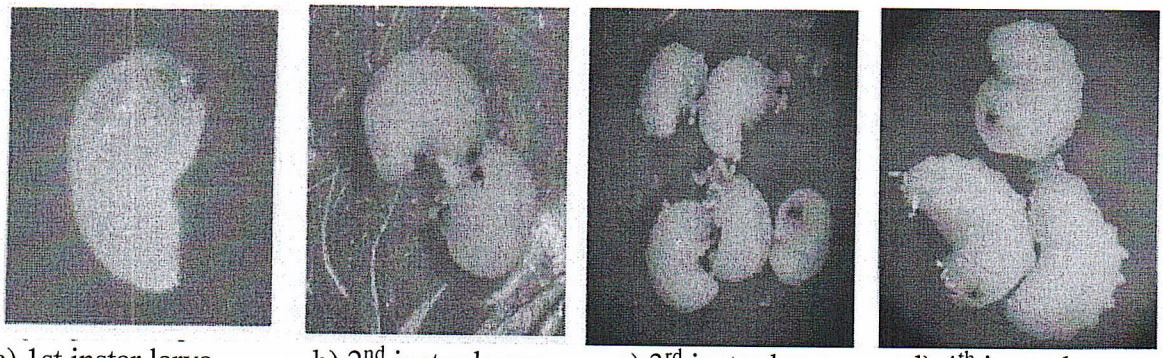
Source: (Devi & Devi, 2014)

Larva: First instar larvae is 0.5mm long. They appear fleshy and white. The oligopod larva has 10 abdominal segments. The 10th abdominal segments bears two bristles of equal size. Second instar larval body are more distinctly arched and about 1.2 mm long. Antennae and legs are more distinct. In the following stages of development the larval instars become more and more arched. Third instar larva is about 1.5 to 1.9 mm long. The fourth instar larvae have complete 'C' shaped body

3/13/2025

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

and a head capsule from where six long and short spines emerges. Fourth instar larva is about of 2.1 to 2.5 mm long (CABI, 2019).

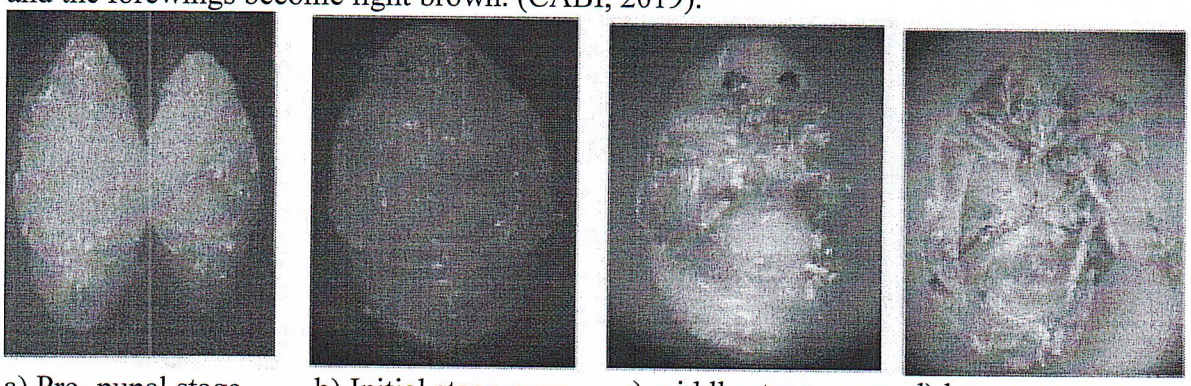


a) 1st instar larva b) 2nd instar larva c) 3rd instar larva d) 4th instar larva

Fig. 2: Different stages of larva of *C. analis*

Source: (Devi & Devi, 2014)

Pupa: The post-pupal stage is about 4–5 days for males and 3–4 days for females. In the second stage pupa, eyes, antennae, proboscis and legs are formed. During the third stage, eyes, mouthparts, forewings, and hindwings are developed, along with cuticular hairs. By the fourth stage, most body parts are fully developed. However, the intersegmental region of the abdomen remains colorless and the forewings become light brown. (CABI, 2019).



a) Pre- pupal stage b) Initial stage pupa c) middle stage pupa d) late stage pupa

Fig. 3: Pupal stage of *C. analis*

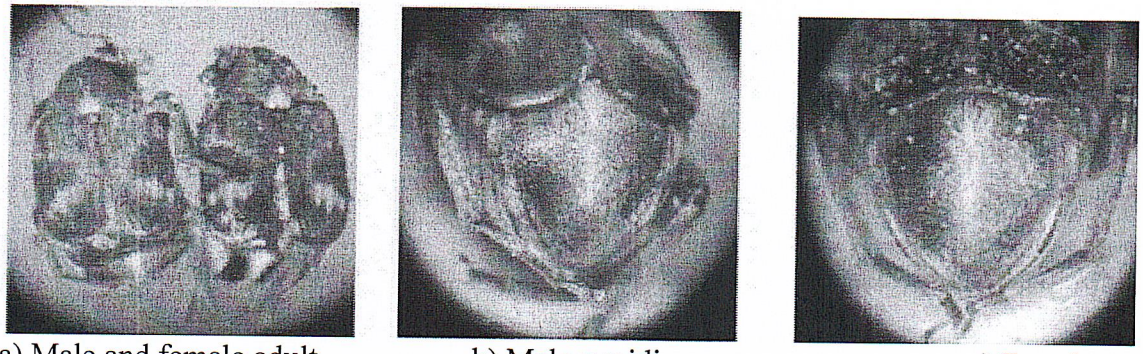
Source: (Devi & Devi, 2014)

The average size of male adult is about 3.2 mm in length and 2.16 mm in breadth while female adult is 3.6 mm in length and 2 mm in breadth (Devi & Devi, 2014). *C. analis* adults have threadlike antennae and red brown pronotum. The adult have distinct dark spots on the middle of the elytra and remaining part of the elytra have smaller and lighter patches covered with numerous clearly visible white setae. The female adults have darker patches on the elytra than the male adults. The lateral side of the female abdomen contains dark spots which is absent in male adults. The outer carina of hind femur (ventral part) has distinct tooth but the inner carina have smaller tooth or sometimes absent (CABI, 2019; Devi & Devi, 2014). The hind femur have numerous tooth but arranged irregularly on the proximal part of hind femur (Seram et al., 2022). The inner tooth in *C. maculatus* and other *Callosobruchus* species is long and prominent from which *C. analis* can be differentiated easily. In Indonesian strains of *C. analis*, the inner tooth and outer tooth may be of equal size. In such case, the species is confirmed by observing male genitalia. The aedeagus is

3/13/2025

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

elongated extending beyond the apex of exophallic valve and of specific curvature (narrowly spatulated) towards their median line that is unique to the species (Devi & Devi, 2014; Seram et al., 2022). The median lobe of **aedeagus** is devoid of scrlerotized region (Seram et al., 2022).



a) Male and female adult b) Male pygidium c) Female pygidium

Fig. 4: Adult stages of *C. analis*

Source: (Devi & Devi, 2014)

4. Mode of dispersal

C. analis is a cosmopolitan pest distributed widely throughout the world. Biogeographically, it is originated in Africa. It later spread to tropical and sub-tropical parts of the world (CABI, 2019). It has polymorphic forms. Adults can fly several distances. Egg, larva, pupa and adult can be dispersed through trade or transportation of infested grains or whole pods.

5. Host range

C. analis is a significant storage pest primarily affecting cowpea (*Vigna unguiculata*) and green gram (*Vigna radiata*). Other legumes also are damaged by this pest such as chickpea (*Cicer arietinum*), black gram (*Vigna mungo*), and bean (*Vigna aconitifolia*), with occasional infestations in pigeon pea (*Cajanus cajan*), lentil (*Lens culinaris*), and various pulses. The geography factor influences its host preferences, which may be due to the availability of legumes. It has also been reported in other hosts of family such as Arecacae, Malvaceae, Asteraceae and Pedaliaceae (CABI, 2019). It has been reported that highest oviposition of the pest occurs in cowpea, pigeon pea and Rajma while progenies were highest on pigeon pea, cowpea, mothbean, chickpea and mung bean (Mannava et al., 2022).

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

3/3/2025

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

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

6. 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:

6.1 Visual inspection method

C. analis can be detected in stored seeds by careful inspection of the seeds. 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 bean or any other host plants should be examined. The packaging and processing areas of medicinal plants should also be included for inspection.

3/31/2025

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

6.2 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. analis,* 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, then *A. analis* 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. The data can be collected and recorded after 3 hours (Njoroge et al., 2019). It can be used along with the food bait for increasing the effectiveness. Depending on the availability on the market, (Z)-3-Methyl-2-heptenoic acid is a synthetic sex pheromone that attracts *C. analis*. However, this pheromone is not available commercially in the market (CABI, 2019). Also the E-noses and electromagnetic spectrum techniques are useful that can be applied inside storage structures. To identify the pest species present in the storage, it gathers, processes, and analyzes images (Banga et al., 2018).

7. Habitat

A. analis can survive in wide range of temperature regimes though its growth and development is somewhat slower in lower temperature, while it survives from cool to warmer areas (CABI, 2022).

8. Reports in Nepal

It has not yet been reported in Nepal. But, it has been reported in neighboring countries like India (Soumia et al., 2015), Pakistan (Shafique & Ahmad, 2005) and Bangladesh (Islam & Khan, 2000). Given the continuous trade of Nepal with other countries, there are chances of entering in Nepal along with import products, with possibility of finding its presence in the detection survey.

9. Purpose

- To detect *C. analis* 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.

10. 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.

3/13/2025

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

Detection Survey Protocol for *Callosobruchus analis* Fabricius in Nepal
NPPO-Nepal, 2025

11. Target pest
The different names of the target pest according to CABI (2019) is given below:

- **Preferred Scientific Name:** *Callosobruchus analis* Fabricius
- **Preferred Common Name**: weevil, bean
- **Other Scientific Names:** *Bruchus analis* Fabricius, *Bruchus ciceri* Rondani, *Bruchus glaber* Allibert, *Bruchus jekelii* Allibert, *Bruchus obliquus* Allibert, *Callosobruchus glaber* (Allibert), *Callosobruchus jekelii* (Allibert).

12. Timing of survey
Time of survey or sampling schedule of *C. analis* is given in Table 1, while, the optimum time for growth and development of the weevil is May-September, hence, .from the initiation to peak period, the adult along with other stages can be detected easily.

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)

13. 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.

14. Design of survey program
14.1 Sampling method
C. analis 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. 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-Nepal, 2024). 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. 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.

3/3/2025

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

14.2 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.

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

15. 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

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

- 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
- 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)

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

16. Collection and preservation of specimen

16.1 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.

16.2 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.
- All the samples should be well-labelled.

16.3 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).

16.4 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

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

3/3/2025

- Family or order of the pest
- Location details
- Collection date
- Name of collector

17. Morphological diagnosis

For successful identification of *C. analis*, 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.
IX. The female genitalia (8^{th} and 9^{th} segment), legs, forewings, antennae should also be prepared in a permanent or temporary slide following the step I to step VIII.

18. Molecular diagnosis

Molecular diagnosis of *C. analis* can be done by several methods such as real time Polymerase Chain Reaction (RT-qPCR), DNA barcoding and DNA sequencing. 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.

19. 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

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

specimen shall be preserved and all records should be securely stored by NPPO (NPPO-Nepal, 2024).

20. Literature cited

Banga, K. M. S., Kotwaliwale, N., Mohapatra, D., & Giri, S. K. (2018). Techniques for insect detection in stored food grains: An overview. *Food Control, 94*, 167-176.

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

CABI Compendium. (2019). *Callosobruchus analis (weevil, bean)*. doi:10.1079/cabicompendium.10985, CABI International.

Devi, M. B., & Devi, N. V. (2014). Life cycle and morphometric measurement of *Callosobruchus analis* on gram. *Biological Forum – An International Journal, 6*(2), 86–89.

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

Islam, W., & Khan, A. R. (2000). Bruchid research in Bangladesh. *Pakistan Journal of Biological Sciences, 3*(1), 10–19.

Mannava, N., Bandi, S. M., Chandra, A., Kumar, V., Aidbhavi, R., Singh, B., & Jambhulkar, P. P. (2022). Bionomics of *Callosobruchus analis* (F.) in ten common food legumes. *Journal of Stored Products Research, 98*, 102010. https://doi.org/10.1016/j.jspr.2022.102010

Njoroge, A., Affognon, H., Richter, U., Hensel, O., Rohde, B., Chen, D., & Mankin, R. (2019). Acoustic, pitfall trap, and visual surveys of stored product insect pests in Kenyan warehouses. *Insects, 10*(4), 105. https://doi.org/10.3390/insects10040105

NPPO-Nepal. (2024). *Technical guidelines for surveillance of plant pests in Nepal*. Plant Quarantine and Pesticide Management Centre.

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

Sarwar, M., Ahmad, N., Bux, M., & Tofique, M. (2012). Potential of plant materials for the management of cowpea bruchid *Callosobruchus analis* (coleoptera: bruchidae) in gram *Cicer arietinum* during storage. The Nucleus, 49(1), 61–64.

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

Shafique, M., & Ahmad, M. (2005). Chickpea grains resistance to pulse beetle, *Callosobruchus analis* (F.) (Coleoptera: Bruchidae). *Pakistan Journal of Zoology, 37*(4), 123.

Soumia, P. S., Srivastava, C., Dikshit, H. K., & Guru Pirasanna Pandi, G. (2015). Screening for resistance against pulse beetle, *Callosobruchus analis* (F.) in greengram (*Vigna radiata* (L.) Wilczek) accessions. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 87*(2), 551–558.

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

3/31/2025

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

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 store

Date:

Host commodity:

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

3/31/2025

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