DETECTION SURVEY PROTOCOL

for *Trogoderma granarium* in Nepal

2022

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

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Detection Survey Protocol for Khapra Beetle (*Trogoderma granarium* Everts, 1898) in Nepal

Endorsed by NPPO Nepal on November 1, 2022

1. Background Information

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 programmes) and ISPM 20 - Guidelines for a phytosanitary import regulatory system). National plant protection organization (NPPO) Nepal has produced the detection survey protocol for *Trogoderma granarium* in Nepal. This will be applicable for monitoring, surveillance, import inspection and export certification. Besides, the purpose of harmonized diagnostic protocols is to support efficient phytosanitary measures. Furthermore these protocols should aid the development of expertise and technical cooperation, and they may also be relevant to the accreditation and/or approval of laboratories.

Under Plant Quarantine and Protection Act, 2064, article 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". This technical guideline to undertake pest detection survey of *Trogoderma granarium* has been prepared with a view to guide the survey activity. This guideline is prepared for the scientists, working in the NARC and/or NAST; plant protection officers, working under the ministry of agriculture and livestock development; professors teaching in the universities or the foresters working under the Ministry of Forest and Environment and other concerned professionals. This document will also guide to transmit specimens to the laboratory for diagnosis and preservation.

Survey is an official procedure conducted over a defined period to determine the presence or absence of pests, or the boundaries or characteristics of a pest population, in an area, place of production or production site [FAO, 1990; revised CEPM, 1996; CPM, 2015; CPM, 2019] (ISPM No. 5 / IPPC Secretariat, 2021). The protocol covers the planning of detection survey program emphasizing the need to carefully document the process.

The Khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae) is recognised as one of the world’s most destructive pests of grain products and is the subject of strict quarantine measures in many countries. Khapra beetle is listed in the “100 World's Worst Invasive Alien Species” by the Global Invasive Species Programme (Lowe et al., 2000). The native distribution of *T. granarium* is not known for certain,

but is believed to be the Indian subcontinent. The beetle occurs in hot, dry conditions, predictably in areas which, for at least 4 months of the year, have a mean temperature greater than 20°C and an RH below 50%. It is especially prevalent in certain areas of the Middle East, Africa and South Asia, and is also found in certain specialized warm habitats in temperate countries e.g. Maltings in the *UK. T. granarium* does not appear to be established in South-East Asia, South America or Australia. Infestations are difficult to control because of the insect's ability to survive without food for long periods, its preference for dry conditions and low-moisture food, and its resistance to many insecticides. Khapra beetle infestation can spoil otherwise valuable trade goods and threaten significant economic losses if introduced to a new area. Handling or consuming contaminated grain and seed products can lead to health issues such as skin irritation and gastrointestinal distress.

Trogoderma granarium Everts (Coleoptera: Dermestidae) is a stored product pest of great importance. Its economic importance lies not only in the serious damage it can cause to stored dry commodities but also in the export restrictions faced by countries when they have established populations of this pest. Live populations can stay in uncleaned containers, packaging material and cargo holds for extended periods of time, infesting non-host material. *Trogoderma granarium* may also increase the likelihood of contamination by Aspergillus flavus (Sinha and Sinha, 1990).

1.1 Taxonomic tree of insect

Domain: Eukaryota
Kingdom: Metazoa
Phylum: Arthropoda
Subphylum: Uniramia
Class: Insecta
Order: Coleoptera
Family: Dermestidae
Genus: *Trogoderma*
Species: *granarium*

1.2 Insect biology

1.2.1 Life cycle and field identification

Trogoderma granarium may have one to more than 10 generations per year depending on food availability and quality, temperature and humidity. A complete life cycle may be as short as 26 days (temperature 32–35°C) or as long as 220 days or more in a suboptimal environment. In temperate climates larvae become inactive at temperatures below 5°C, so the pest is able to survive and breed only in protected environment.

These are important features of the pest that let to survive in harsh conditions as well. There are two genetic variations of larvae: those that are able to undergo facultative diapause and those that are unable to do so. Larvae of the first type are stimulated into diapause by adverse conditions such as low or high temperatures and/or lack of food. During diapause their respiration drops to an extremely low level leading to tolerance to fumigation. Diapausing larvae are also cold-hardy and may survive temperatures below 10°C. When favourable conditions return, the pest is able to multiply rapidly and cause serious damage to the commodity (EPPO/CABI, 1997).

Mating between adult male and female beetles occurs around 2–3 days after emergence. Shortly after mating, adult females begin laying eggs in or near host material and generally lay 50–100 eggs during their lifetime. Eggs hatch within 5–7 days into larvae, which are the major feeding stage and therefore the most damaging. Larvae moult four or more times, resulting in numerous cast larval skins. The larval development period can be as short as 30 days, but larvae can also survive in a dormant state for several years in unfavourable conditions. The larva finally develops into an immobile pupa, from which the adult emerges.

(Source: California Agriculture, 1954)

Eggs of Khapra beetle

Adults are short-lived, do not feed much, and although they are winged, they are not known to fly. There can be up to ten generations per year during warm, dry conditions, which can quickly result in damaging infestations.

1.2.1.1 Eggs

Eggs are less than 1mm long, cylindrical, one end rounded and other end pointed with spine like projections. They are milky white and turn pale yellow in colour. Eggs are laid on the surface of grain and other stored products.

1.2.1.2 Larva

Mature larvae are 4.0-6.0 mm long, yellowish brown golden brown in color, and have distinctive reddish brown hairs across the body, including longer hairs at the end of the body that resemble a tail. Larvae have 5 to 11 instars in stored products and may be found in packing material or within storage structure. Under unfavorable conditions (overpopulation, poor-quality food, a large amount of excrement), the larvae enter a state of diapause. They try to hide in narrow places, crawl into cracks in walls, pillars, behind

plaster. Here they stop eating, move, slow down breathing and can stay in this state from several weeks to 4 years. Such larvae are extremely resistant to both low and high critical temperatures, as well as to pesticides.

(Source: Arakelian, 2013)

Larva – Dorsal View

Khapra beetle larvae inside cardboard packaging corrugations.

Heavy infestation under the flooring of a shipping container comprising larvae and larval skins

1.2.1.3 Pupa

Pupae have a similar appearance to late stage larvae except that they are slightly shorter and more rounded. Pupae are found in stored products.

1.2.1.4 Adult

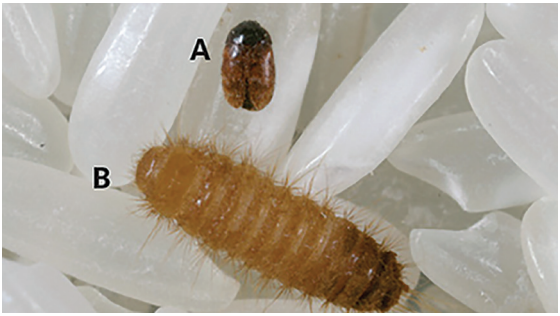
Adults are 1.6–3.4 mm long; oblong-oval shaped and light yellowish brown to dark brown in color. Adult beetles have many fine hairs and indistinct reddish brown markings on their wing covers. When viewed from the side, the adult beetle's partly

Adult khapra beetle (side view)

concealed head and downward facing mouthparts can be seen. Females are noticeably larger than males. Adult's life span may vary between 12-25 days on an average.

1.2.2 Mode of Dispersal

T. granarium has very limited ability to spread without human aid because it is unable to fly, so international movement of host commodities appears to be the only means of spreading the pest. It is very important to distinguish between records that relate to interceptions of the pest in imported commodities (i.e. its finding in the commodity during the border phytosanitary control without further spread) and those of established infestations (EPPO, 2011).



Khapra beetle adult (A) and larva (B) on grains of rice

(Source : Arakelian, 2013)

Adult-Dorsal View

1.2.3 Host range

T. granarium usually occurs in various dry stored products of primarily plant origin. Main hosts are barley, cotton, groundnut, maize, millet, rice, sesame, sorghum, and wheat (CABI, 2021). It can also successfully complete its life cycle in copra, dried fruits and various gums, as well as many different dried products wholly or partially of animal origin, such as milk powder, skins, dried dog food, dried blood, dead insects and dried animal carcasses.

1.3 Detection

1.3.1 Detection through cast larval skins (exuviae)

T. granarium infestations are usually recognized by (1) the presence of the pest (especially feeding larvae and exuviae) and (2) symptoms of infestation. In bag stores, the first signs of infestation are masses of hairy cast larval skins push out from the crevices between sacks. Larvae crawl over and consume the grain. Larvae usually feed first on the germ portion of cereal seeds and then on the endosperm. The seed coat is

eaten in an irregular manner. In bulk commodities infestations usually concentrate in the surface layers, where numerous larval exuviae, broken setae and frass (excrement) are present. However, larvae can occasionally be found as deep as 3–6 m in bulk grain. It is therefore important to consider biased sampling when inspecting for these types of pests. The short-lived adults are sometimes not seen. Damage to the commodities can be a warning sign, but often it is a result of the feeding of other common stored product pests.

Detection methods include examination of cracks and crevices and inspecting behind paneling on walls and under timbers, tanks, shelves, etc. Infestations are usually spotted by accumulations of cast larval skins. In storage facilities, trapping using pheromone and larval traps has proved to be a useful surveillance tool.

1.3.2 Detection through a stereoscopic microscope and a set of Berlese funnel (especially in case of heavy infestation)

Samples of suspect products have to be visually inspected in a well-lit area, using a 10x magnification hand lens. If appropriate, samples should be passed over sieves with aperture sizes relevant to the particle size of the products. Usually sets of sieves of aperture sizes 1, 2 and 3 mm are used. The sifted material collected on particular sieves should be placed in petri-dishes and examine the specimens through a stereoscopic microscope to detect the pest. This screening technique allows the detection of various developmental stages of the pest. However, some larvae feeding within grains may remain undetected. Therefore, it may become necessary to heat samples to 40 0C to drive pests out of the grains with an extractor tool such as a Berlese funnel, especially in case of heavy infestation. Visual inspection is preferable to sieving because the latter can easily destroy or seriously damage dead adults and larval exuviae rendering the morphological identification very difficult or impossible.

1.3.3 Detection through insect attractants

1.3.3.1 Larval food bait trap for attracting and trapping

Additionally to initial inspections, it is possible to monitor the presence of *T. granarium* using various traps. Food-baited traps (containing oil seeds, peanuts, wheat germ etc.) or attractant traps (containing wheat germ oil) can be used to attract larvae. Simple traps offering hiding places for the larvae, such as pieces of corrugated cardboard or hessian bag, can be placed on the floor. After monitoring, all the traps should be destroyed. Adults may be detected with the use of pheromone traps where the pheromone capsule is combined with a non-drying sticky trap.

1.3.3.2 Pheromone trapping of male beetles

The pheromone for *T. granarium* includes a mixture with 92% of the Z, and 8% of the E isomer of 14-methyl-8-hexadecenal (an aldehyde). Generally, pheromone + wheat germ oil (food attractant) on sticker put on floor to trap male beetles and active larvae, at the same time, in the grain storage.

However, the *Trogoderma* pheromone traps are not species-specific and attract many species of dermestid beetles (Saplina, 1984; Barak, 1989; Barak et al., 1990; Mordkovich & Sokolov, 2000). Traps baited with both the pheromone and food bait traps are commercially available.

1.4 Habitat

Some types of properties are more likely to harbor khapra beetle. Inspection should be carried out in the properties within an area according to the following order of importance.

- Distributors of host material; spice importers, markets, brassware
- Grain dealers
- Feed lots
- Users
- Farm storages

Probable hiding niches of *T. granarium*

- Cracks in walls and floors
- Behind loose paint or rust
- Along pallet, and the end-grain of pallet wood
- Seams and ears of burlap bags
- Low light areas
- Trash from cleaning equipment, and the equipment itself.

1.5 Occasional reports of *Trogoderma granarium* in Nepal

According to the NARC publication, *T. granarium* was noticed in Nepal as it reported from Pokhara valley in the western region of Nepal in stored grains in 1976 (Joshi and Manandhar, 2001). Further, GC (2001) listed *T. granarium* in maize grain pests, in other occasion; GC (2006) scribed its name in a scientific paper. Additionally, the US Customs and Border Protection intercepted live *T. granarium* larvae in beans from Nepal in 2016.

2. Detection Survey

Nepal has been identified as a Khapra beetle (*Trogoderma granarium*) target risk country by the Australian Government, Department of Agriculture, Water and the Environment.

As such, to comply with the new measures, all high-risk plant products exported as commercial sea and air freight from Nepal on or after 28 April 2022 must be:

Treated offshore using an approved treatment option of either methyl bromide fumigation, heat treatment or modified atmosphere treatments (alternative treatment options)

Inspected offshore by government official of exporting country;

Certified as being free from any listed species of Trogoderma (whether live, dead or exuviae) in Australia's list of Trogoderma species of biosecurity concern.

In addition, the additional declaration on the Phytosanitary Certificate must include "Representative samples were inspected and found free from evidence of any species of Trogoderma (whether live, dead or exuviae) in Australia's list of Trogoderma species of biosecurity concern".

Above all, this insect has been reported in 1976 in Nepal but its spread in the country is in doubt.

2.1 Purpose of detection survey

To detect the presence of *Trogoderma granarium* in Nepal.

2.2 Scope

The scope of detection survey of *T. granarium* covers all the provinces of Nepal where the pest is supposed to survive based on the optimum prevailing temperature range between 30 to 40^{0}C in terai and inner terai region.

2.3 Target pest

Preferred Scientific Name

- *Trogoderma granarium* Everts

Preferred Common Name Khapra beetle

Other Scientific Names

- *Trogoderma khapra* Arrow
- *Trogoderma koningsbergeri* Pic.
- *Trogoderma affrum* Priesner
- *Trogoderma granarium* ssp. afrum Attia and Kamel

Common Names

- **English:** beetle, khapra, cabinet beetle
- **Spanish:** *dermeste de los granos; escarabajokhapra; gorgojo de khapra; gorgojokhapra*
- **French:** *dermeste des grains; trogoderme; trogoderme des grains*
- **Nepali:** KHAPRA KHAPATE KIRA

2.4 Timing of survey

Timing of the survey for the detection of Khapra beetle is presented in the table 1.

2.5 Area or site selection

Site of survey for the detection of Khapra beetle is presented in the table 1.

Table 1. Commodities, their storage structures and timing of sample collection

Commodities	Type of storage structure				Timing/duration replication				Sampling bag
	Ware-house	DHANSAR (wooden st.)	BHAKAR/ DEHARI	Gunny bags					
Rice	upper surface	upper surface	upper surface	upper surface	May	Jun.	Jul.	Aug.	Zip lock plastic bag
Wheat	upper surface	upper surface	upper surface	upper surface	May	Jun.	Jul.	Aug.	Zip lock plastic bag
Maize	upper surface	upper surface	upper surface	upper surface	May	Jun.	Jul.	Aug.	Zip lock plastic bag
Groundnut	upper surface	upper surface	upper surface	upper surface	May	Jun.	Jul.	Aug.	Zip lock plastic bag

Sampling of stored grain should be from upper surface in between 4-10 cm randomly picked up quantity. Zip lock high density plastic bag (500 gm) will be used to collect sample from the storage structures. The collected samples will be kept in sample collection jar.

2.6 Location of Survey

Based on the previous records and the potential of introduction of the pest along with the commodities, the terai and valleys from all the provinces will be covered in this survey. Warehouses, mills and the breweries will be observed for the presence of the pest and the samples of the commodities will also be collected for observation. The survey districts decided by NPPO Nepal presented in the annex 1. The survey data will be collected as per the format in annex 2.

3. Design of survey program

3.1 Introduction

Detection survey is conducted in an area to determine the presence or absence of pest. *Trogoderma granarium* has the following life developmental stages: eggs on the surface of grain and other stored products; larvae (5–11 instars) in stored products (larvae may be found in packing material or within storage structures); pupae in stored products, in the last larval exuviae (cast skins); adults in stored products.

Methods to detect *T. granarium* infestations include inspection, physical search, use of food baits and pheromone traps. Often the infested material contains only larvae because (1) adult longevity is usually between 12 and25 days (it can be as long as 147 days in unfavourable conditions), whereas larval longevity is usually 19–190 days (and can be up to 6 years in diapausing larvae); (2) most of the dermestid larvae occurring in stored products will partially or wholly consume dead adults; and (3) adults are most prevalent when conditions are favourable for population growth. Larval exuviae are usually not consumed so their presence is a clear indication of a possible active infestation. Larvae are extremely cryptic by nature, particularly diapausing larvae that may stay inactive for long periods in cracks and crevices where they are very difficult or nearly impossible to locate.

3.2 Methodology

Inspection should start on arrival at the premises or location. Exit and entry areas should be checked, as well as any storage locations on the premises. The movement of products, containers, or people handling such products, which could have been exposed to khapra beetle, should be observed.

Observation should be on:

- Cartons, sacks, debris, woodwork, cracks, loose plaster, loose paint, and other such hiding places.
- Milled products or debris from areas such as cracks and crevices of bins or silos or wherever grain is stored or emptied, such as in farm buildings, homes, stores, etc.
- In bulk storage where heavy infestations occur, the larvae tend to congregate in the surface grain and on or near the walls.
- In empty bins and warehouses, likely places to find larvae are in or on ledges, cracks in the floor or walls, old cartons, rags, sacks, newspapers, and scrap lumber, or other debris.

3.3 Materials required for survey

Knife,
Gloves,
Illuminated magnifying lens (10X),
Torch,
Scissors,
Sample containers/bags,
Zip locks plastic bags,
Sample collection jar,
Camel hair brush,
Tweezers or forceps,
Vials,
Petri dishes,
Tags,
Permanent markers,
Rubber bands,
Collecting/killing jars,
Absolute alcohol,
Aspirator,
GPS,
Thermo-hygrometer (data-logger),
Camera,
Data sheets
Color book
Pheromone lure and trap
(Khapra Beetle Wall Mount Trap Kit with khapra beetle pheromone lures)
Food lure and trap (Organic wheat germ - the food bait for the beetle)

3.4 Collection and preservation of specimens

Insects found should be picked up carefully with camel hair bruss or collected using an aspirator. It is important to collect multiple specimens of the pest. Identification of larvae is difficult, if the dissection of a single specimen is not successful and serious damage occurs to the mouthparts, exact identification is impossible. Larvae specimen should be reared for further identification.

Trap setting and observation

Trap:
Trap density:
Owner:
Sampling date:
Sampling time:
Sampling unit: 250 g
Locality name:
Altitude of the locality:
GPS coordinates of locality:
Existing temperature 0C:
Existing air Relative Humidity %:

S N	District	Warehouse	No. of traps	No. of observation	Insect population per trap

Specimens should be placed in 70% ethyl alcohol for preservation and safe shipping if the identification is not done immediately at the same locality.

3.5 Procedure for preparation of larvae and larval exuviae

Before dissection the larva should be examined under a stereomicroscope. Size, body colour, arrangement and colour of setae should be recorded. Use of microscope photography provides a record of material prior to disturbance via manipulation and handling and so allows for its independent interpretation.

For identification the larvae should be mounted in Hoyer's medium or other mounting media such as PVA on a microscope slide using the following method:

1. First, place the specimen on a microscope slide; it is best done ventral side up in order to preserve the diagnostic characters.
2. Cut open the whole body along the mid-line from under the head capsule to the last abdominal segment using eye surgery scissors.
3. Next put the larva into a test-tube containing 10% potassium hydroxide (KOH) solution and heat in a boiling water bath until larval tissues loosen and begin to separate from the cuticle.
4. Rinse thoroughly in warm distilled water.
5. Remove all internal tissues using a very fine, short hair brush or the convex surface of a hooked tip of a no. 1 insect pin, or a loop formed from a micropin. All setae

should be removed from one side of the 7th and 8th abdominal segment; stains such as acid fuchsin or chlorazol black may be used to make the analysed structures more visible.

6. Remove the head capsule and put it back in the hot KOH solution for 5 minutes. Rinse the head capsule in warm distilled water. Dissection of the head can be performed in a few drops of Hoyer's mounting medium or glycerol on a microscope slide or in water in an excavated glass block. Turn the head ventral side up and hold it to the glass with a blunt no. 1 insect pin.
7. Remove the mandibles, maxillae and labial palpi using jeweller's forceps and micropins. Remove the epipharynx and antennae, which may be additionally stained with a stain such as acid fuchsin or chlorazol black. Mount the head capsule and the mandibles in the cavity of the slide using Hoyer's medium or another mounting media. Mount the cleared skin, fully opened on the flat part of the microscope slide, next to the cavity. It is usually best done ventral side up. Epipharynx, antennae, maxillae and labial palpi should be mounted with the skin under the same cover slip. Mount all body parts on the same microscope slide.
8. In the case of larval exuviae, before proceeding with the dissection soak the specimen in a 5% solution of any laboratory detergent for about two hours and rinse thoroughly in distilled water. Cut the specimen open anteriorly and dissect out the mouthparts. They can be mounted directly in Hoyer's medium without clearing.
9. Label slides immediately after mounting specimens and place them in an oven for at least three days at 40 °C to improve their quality (the best slides are obtained after 2–4 weeks). After drying, ring the slides using any lacquer recommended for sealing of microscopic slides (e.g. Glyptal, Brunseal), or at least two layers of nail polish in order to prevent the Hoyer's medium from drying and possibly damaging the specimen. However, microscopic slides may be examined immediately after preparing. Permanent slides can be made using Euparal or Canada balsam for mounting, but these require a laborious dehydration process.

3.6. Procedure for preparation of adults

Adult Trogoderma specimens may need to be cleaned before identification, with any laboratory detergent or using an ultrasonic cleaner. If the specimen was caught in a sticky trap the glue can be dissolved using a number of solvents (e.g. kerosene). These solvents can be removed from the specimen with any laboratory detergent.

Before beginning the preparation, soak the adult in warm distilled water for about an hour. Perform the preparation in the following way:

1. First remove abdomen while the specimen is still in the water using fine forceps. Dry the specimen (minus abdomen) and mount it on a cardboard rectangle, preferably

laterally. The specimen will be less exposed to damage and accessible for both dorsal and ventral examination if it is glued on the side.

2. Next cut the abdomen laterally open, leaving the last abdominal segment untouched. Place it in a 10% KOH or sodium hydroxide (NaOH) solution in a hot water bath for about 10 minutes.
3. Rinse the specimen in water and carefully remove the genitalia using hooked micro pins. After removing the genitalia the abdomen should be glued onto the same cardboard rectangle with the insect, ventral side facing up.
4. The genitalia need to be macerated further in the caustic solution. Separate the aedeagus from the periphallicter gum and the 9th abdominal segment using micro pins. They may be stained with a stain such as acid fuchsin or chlorazol black to make them more visible. Genitalia can be mounted on a microscope slide using Hoyer's medium or other mounting media such as PVA. The aedeagus should be mounted on a cavity microscope slide to keep its shape. Female genitalia can be mounted on a flat microscope slide. Slides and pinned insects should be labelled immediately after mounting the specimens. The slides should be placed in an oven for at least three days at 40 ºC (the best slides are obtained after 2–4 weeks). After drying, all slides should be ringed.

If there is no need for mounting the genitalia using a permanent or semi-permanent mounting agent, they can be examined in a drop of glycerol on a microscope slide. After the identification the organ scan be placed in a microbial in a drop of glycerol or glued onto the cardboard rectangle next to the abdomen.

3.7 Sample analysis and reporting

Concerned laboratory, if analyses and identifies the specimen, should submit the report to the NPPO. If the specimen is analysed and identified by National Entomology Research Center or Central Agricultural Laboratory or any other institution, they should report to NPPO for the reporting/declaration of insect-pest.

3.8 Record keeping

NPPO, in collaboration with responsible laboratories, should preserve the specimen and keep all the record safely. The documentation system should be well maintained by the NPPO and member institutions should have easy access to it.

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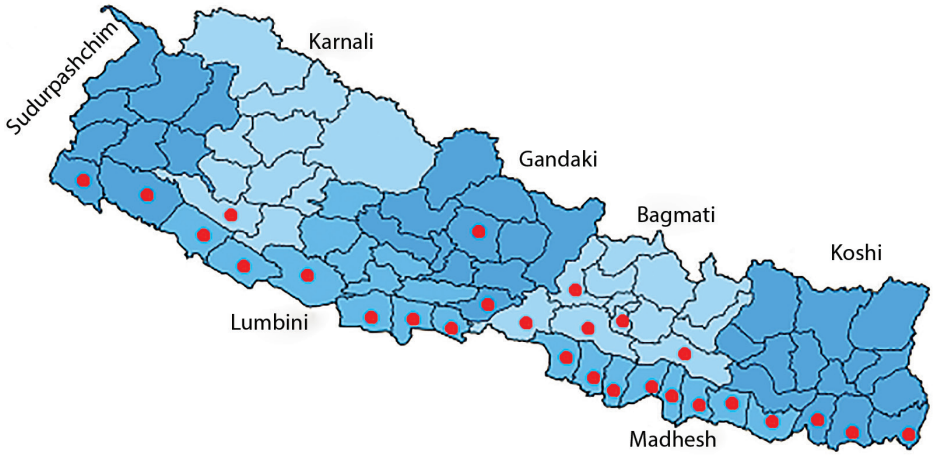
Annex 1. Selected districts for the detection survey of khapra beetle in Nepal

No. of districts in a province	Agro-ecological zones			Total	Khapra beetle potential districts			Total	Sample districts			Total
	Mt.	Hills	Terai		Mt.	Hills	Terai		Mt.	Hills	Terai	
Koshi	3	8	3	14	0	0	3	3	0	0	2	2
Madhesh	0	0	8	8	0	0	8	8	0	0	4	4
Bagmati	3	9	1	13	0	4	1	1	0	2	1	3
Gandaki	2	8	1	11	0	1	1	1	0	1	1	2
Lumbini	0	6	6	12	0	0	6	6	0	0	3	3
Karnali	5	5	0	10	0	1	0	0	0	0	1	1
SudurPaschim	3	4	2	9	0		2	2	0	0	2	2
Total	16	40	21	77	0	6	21	27		3	14	17



Khapra beetle potential districts		Proposed districts for F/Y 2078/79:
Jhapa	Makwanpur	Morang
Morang	Nawalparasi	Parsa
Sunsari	Nawalpur	Chitwan
Saptari	Kaski	Nawalpur
Dhanusha	Chitwan	Rupandehi
Siraha	Bardiya	Palpa
Mahottari	Banke	Banke
Sarlahi	Dang	Kailali
Rautahat	Kapilbastu	
Bara	Rupandehi	
Parsa	Surkhet	
Dhading	Kailali	
Sindhuli	Kanchanpur	
Lalitpur		

Annex 2. Sampling record sheet for detection survey of khapra beetle

Storage structure:
Owner:
Sampling date:
Sampling time:
Sampling unit: 250 g
Locality name:
Altitude of the locality:
GPS coordinates of locality:
Existing temperature oc:
Existing air Relative Humidity %:

Stored commodity	Storage period / duration	Status of commodity			Treatment		Pest observed		Remarks
		Whole	mechanical-broken	insect damaged	Yes (if yes, specify)	No	Yes (if yes, specify stage)	No	

Note: Status of whole grain-ness of commodity in unit sample (250 gm) will be assessed by dividing into 4 parts and 2 parts will be randomly picked up and will be mixed. Thus, obtained sub-sample part will be divided again in 4 parts and it's randomly selected 2 parts will be again mixed and sub divided and randomly taken one part of it will be used to obtain the status of whole grain-ness of the commodity.

NOTE

NOTE