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Detection survey protocol for *Oxalis latifolia* Kunth. in Nepal

Agriculture and Livestock Development

Plant Quarantine and Pesticide Management Centre

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1. Background information

With entry in the WTO, Nepal gets the opportunity to export its produce to the international markets. However, the exports from Nepal have not escalated to the same proportion as trade between developed nations. The developed countries have increased exports by using the rules of the SPS Agreement. At the moment, the Government of Nepal is obliged to use the SPS rules to exclude commodities that are posing threat to the related industries within the country. Nepal should provide adequate description of the health status of plant based industries, while negotiating access to foreign trades. Prospective importers of Nepalese agriculture related commodities assess risk of introducing new pests based on the authentic pest information provided. Prospective importers also assess the phytosanitary measures being practiced in Nepal to reduce risk to an acceptable level. Extensive specimen-based records are the key for Nepal to negotiate with importing countries on a fair trading system. This document gives detailed guidelines for detection surveys of the noxious and invasive weeds *Oxalis* in the agriculture field. Besides, it will be applicable for monitoring, surveillance, import inspection and export certification and is the basis for specimen based records to be developed by the NPPO-Nepal.

Under Plant Quarantine and Protection Act, 2064, article 6(2), survey and surveillance function and responsibility is designated to NPPO-Nepal as per the sub clause (i) "To perform such other function as prescribed". This technical guideline to undertake pest detection survey of *Oxalis latifolia* has been prepared with a view to guide the survey activity. This guideline is prepared for researchers, plant protectionists, teachers, and other concerned professionals. This document will be a guide to submit specimens to the laboratory for diagnosis and preservation.

1.1 About the pest (weed)

The genus *Oxalis* Kunth. (Sorrel) comprises approximately 850 species of small herbaceous plant, native primarily to southern Africa and tropical and South America. It is mainly distributed in tropical and subtropical regions, and also in temperate regions of the both new and old world. Some species cause oxalate poisoning when animals ingest large amounts of plants, especially those with the more toxic potassium acid oxalate (with very acid cell sap, pH=2). Among several *Oxalis* species, *Oxalis latifolia* (Kunth) (1753), known as broadleaf woodsorrel, is a low-growing plant with three broad leaflets on long petioles. It is a perennial herbaceous plant which has caused losses of 30 different types of crops, including rice, tea, potato and apple, in 37 countries (Holm et al., 1997; Royo-Esnal & López, 2008; Shrestha et al., 2019). In Nepal, it is found in 600-2200m altitude and reported first time in 1954 (Adhikari et al., 2022). It absorbs a relatively high proportion of soil nutrients and moisture and creates an allelopathic effect, inhibiting the growth of surrounding plants (Thomas, 1991). The weed also has a significant impact on nurseries, resulting in a loss of garden plants. Based on severity, the crop yield loss due to *Oxalis* infection has been estimated to be about 56% in maize (Atwal and Gopal, 1972). *O. latifolia* is an alternate host of *Puccinia sorghi* (CABI, 2021). In account to the seriousness of the invasive and noxious weed, implementing a thorough survey and surveillance technique is essential to precisely tracking and managing the spread of *Oxalis*. The development and implementation of an effective protocol would facilitate detecting early infestation, prompt response, and the implement of control measures – all of which are essential for ensuring sustainable exports of haylage from Nepal to China on the basis of Pest Risk Analysis (PRA) of

the World Trade Organization Agreement on the application of sanitary and phytosanitary measures (WTO SPS). The spread of seeds mostly occurs due to increasing trade, travel and other accidental introduction. Because the probability of introducing the weed from infested to other non-infested countries is high, a vigilant approach to surveillance and quarantine is required. This protocol provides guidelines for planning and conducting *Oxalis* survey and surveillance activities.

1.2 Identity and taxonomy of target pest (CABI, 2021)

1.2.1 Identity

Preferred scientific name: *Oxalis latifolia* (Kunth)

Preferred common name: Broadleaf wood sorrel or garden pink sorrel

Other scientific names: *Acetosella violacea* subsp. *latifolia* (Kunth) Kuntze, *Ionoxalis latifolia* L., *Oxalis violacea* L., *Sassia latifolia* (Kunth) Holub.

Nepali local names: Thulo Chari amilo

EPPO code: OXALA (*Oxalis latifolia*)

1.2.2 Taxonomy

Taxonomic tree of the *Oxalis* is presented below

Kingdom:	Plantae
Phylum:	Spermatophyta
Class:	Dicotyledonae
Order:	Geraniales
Family:	Oxalidaceae
Genus:	*Oxalis*
Species:	*Oxalis latifolia*

1.3 Host range

The main host are: Maize (*Zea mays*), Sugarcane (*Saccharum officinarum*), Rice (*Oryza sativa*), Soyabean (*Glycine max*), Tomato (*Solanum lycopersicum*), Potato (*Solanum tuberosum*). Onion (*Allium cepa*), Groundnut (*Arachis hypogea*)

The *Oxalis* are occur in a very wide range of crops in the tropics and subtropics and it perhaps of greatest importance when it infests ornamental nurseries and contaminates stock that is sold. Hence the host range can include a vast range of fruit and ornamental species, annual and perennial, as well as many vegetable crops.

1.4 Weed biology

Oxalis latifolia is a plant of humid tropics, especially at higher elevations (upto 3000m in Colombia) and of the sub-tropics. It is a bulbous perennial, spreading vegetatively by bulbs and rarely producing seeds. In most countries, it is not thought to set seed at all, but Rivals (1960) notes that it does so in France. It apparently has the potential for tristyly, having two sets of five stamens of different length. Weedy populations are almost invariably short-styled, though one of the

atypical, rounded-leaved forms in New Zealand is reported to be mid-styled (Esler, 1962). The behaviour of the bulbs of *Oxalis latifolia* has been the subject of several studies. Most bulbils remain dormant while still attached to the parent plant, though Marshall and Gitari (1988) note that the first bulbils to be produced usually develop one or more leaves while still attached. Factors involved in breaking the dormancy of the bulbs include chilling (5°C for 3 weeks) and dry heat (45°C for several hours) (Chawdhry and Sagar, 1974). Dormancy can last for more than one year (Holm et al., 1997). In a study by Esler (1962) all bulbs emerged from 8 cm depth and 20% from 20 cm. On sprouting, the bulbs produce a ring of adventitious roots, one of which later becomes the main fleshy taproot. Petioles are then produced from the inner membranous scales. Stolon development follows the formation of the taproot. Plants growing from bulbils may not flower in their first or even second season of growth but develop gradually larger bulbs. Eventually, peduncles develop from the axils of the outer membranous scales. The parent bulb disintegrates at the end of a growing season but is replaced by a new main bulb, which may draw on the taproot for resources as the latter shrivels. O. *latifolia* occurs on a wide range of soil types. The bulbs survive short periods of freezing but are killed by prolonged exposure to sub-zero temperatures. The schematic diagram of *Oxalis latifolia* presented in Figure 1.

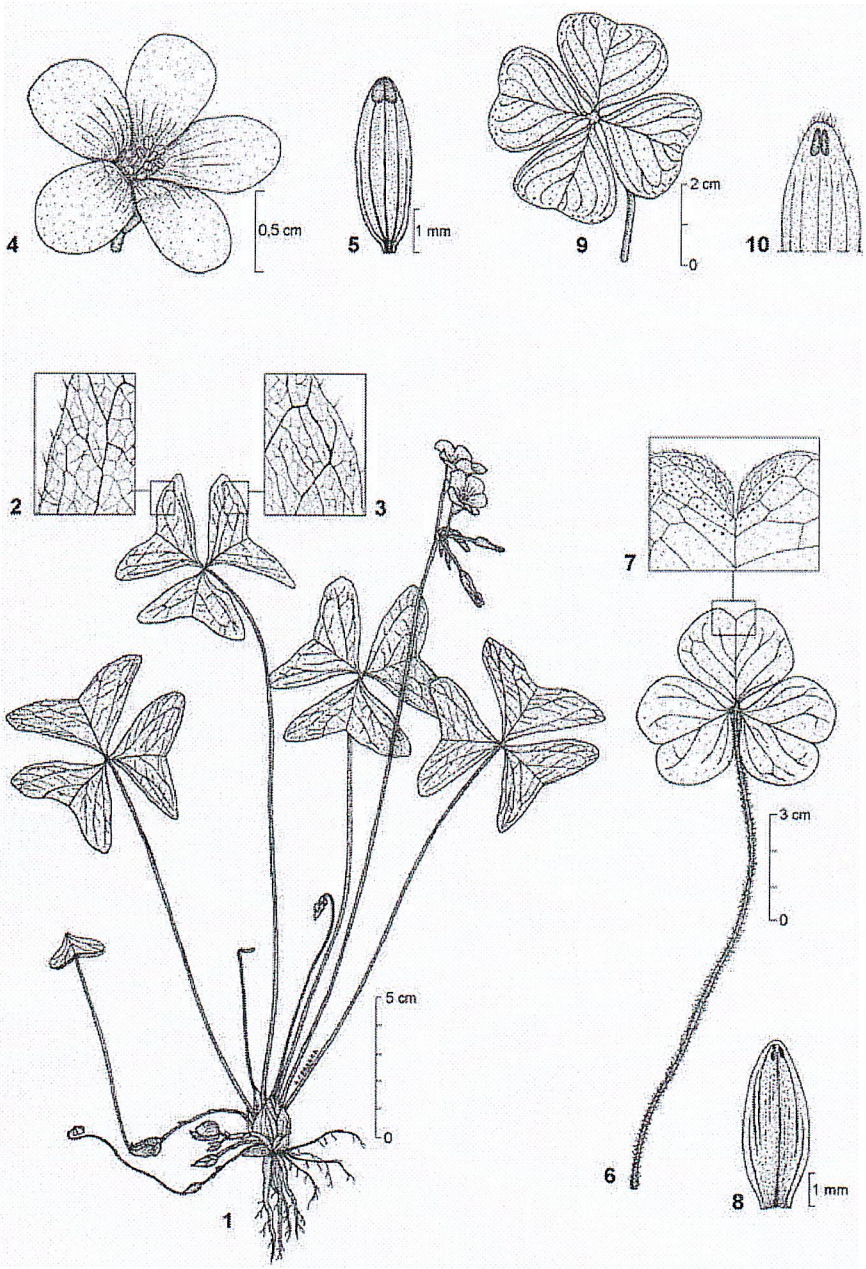

Figure 1. Schematic illustration of the *Oxalis latifolia* (Source: *https://portal.wiktrop.org/species)*

1.5 Mode of dispersion

The primary mode of dispersion of *Oxalis latifolia* occurs through bulbils and seed dispersal mechanisms (CABI, 2021). Some of the modes of its dispersion are:

a) **Natural dispersal:** *Oxalis latifolia* has underground bulbils which can root at the nodes and thus spread the plant clonally. These bulbils detach from the parent plant and produce new plants, allowing the species to form dense colonies. The seeds naturally disperse considerable distance (up to 2 m) from the parent plant during dehiscence of the capsule.

b) **Animal dispersal:** Seeds may adhere to the fur or feathers of animals, facilitating transport to new locations. The seeds may be carried out by birds.
c) **Human activities:** Movement of soil containing bulbils or seeds can contribute significantly to its spread, especially during agricultural activities or gardening. Bulbils and seeds may also be transported unintentionally through contaminated equipment or vehicles.

2. Detection survey

Detection survey is conducted in an area to determine if pests are present (FAO, 1990; revised FAO, 1995). These surveys are more frequently carried out to determine pest status in an area and they follow a definite survey plan, which is approved by NPPO-Nepal. These surveys are carried out either seasonally or annually and/ or following the eradication measures applied to a pest in a given area or production sites. These surveys are organized following a definite survey methodologies based on statistical sampling, which are determined after taking into account the biology of the pest and employing appropriate detection techniques such as field diagnostic kits, traps etc. The results of the survey are documented and communicated (PPD/NPPO-Nepal, 2071 BS).

2.1 Purpose and scope of detection survey

The purpose of the detection survey is to determine the presence or absence of *Oxalis latifolia* in a given area or production sites. The scope will be limited to maize and other defined crops to be grown for haylage/silage production for export to China and other concerned countries.

2.2 Timing of survey

The timing of survey should be coincided with the flowering period as the plant species is generally identified by morphology and flower characteristics. Flowering often occurs during the late winter or early spring period (February-April) in Nepal. Detection surveys need to be done during the pre-planting, vegetative stage and reproductive stage of the host crop (*i.e.*, maize) for detection of *Oxalis latifolia* in the field.

2.3 Selection of survey area

Field plots of maize and concerned crops in the target areas.

2.4 Materials required for survey

- Field press bag
- Herbarium press (45×30 cm)
- Secateurs and scissor
- Plant digger (hand hoe or shovel)
- Gloves, scales and ropes
- Zip locks plastic bags (18×41 cm)
- Newspaper and Blotting sheet
- Topographic maps
- Altimeter
- GPS or mobile apps with geotagging
- Magnification glass
- Forceps, needle, brush and glue
- Field notebook, logbooks

- Permanent ink pens and tags
- High pixel digital camera
- Field guide with image of *Oxalis latifolia*
- Mini glass/plastic vials for weed seeds
- Chemicals (preservatives), if necessary

2.5 Identification method

Classification and identification of *Oxalis* species depends largely on floral characters. Morphological identification of *Oxalis* plants (including seeds) is based on known reference specimens, literature descriptions and taxonomic keys and descriptions. Considerable data from molecular studies of *Oxalis* are available and can be helpful for species determination, but until methods can be simplified and made more uniform they are of limited value for phytosanitary purposes.

2.5.1 Identification of mature plants

Morphological characteristics of mature plants of the *Oxalis* species are summarized in Table (2). *Oxalis latifolia* is stemless grass, which is in the form of a small tuft, 20 to 30 cm high, with trifoliate triangular, long-stalked leaves and with long stalked inflorescences (Figure 5). The underground system is waxy white taproot, with well-developed secondary formations, along with some rootlets. Globular or ovoid bulb, reaching up to 2.5 cm long and 2 cm wide. It is surrounded by large papery protective scales, and brown feeder scales, elliptical, narrow from 7 to 15 mm long and 4 to 6 mm wide (Figure 4). Stipules translucent to papery, red, with ciliated and glandular margins, with orange granulations. Rhizomes emerging from the base of the bulb carries white bulbils at the end. The aerial stem is absent. Underground stem reduced to the axis of the bulb. The first leaves are trifoliate, long-stalked, emerging from a scaly bulb. Notched or triangular leaflets with wedged base and flat or notched, divergent apex, having orange granulations. Fasciculate leaves in tuft, compound, with three terminal digitate leaflets, carried by a vertical petiole of 5 to 20 cm long. Green leaflets, purplish beneath. The blade is entire, sub sessile, obtriangular or notched at the top, 1 to 5 cm long and 2 to 7 cm wide. The base is wedged, the apex is narrow or notched, sometimes mucronate. The leaf blade is more or less folded along the midrib. Both sides are glabrous or sub glabrous, with the presence of orange granulations at the base of the lower face (Figure 2). The inflorescence is an umbelliform cyme, often bifid, carried by a long peduncle, 10-25 cm long, carrying 5-12 flowers (Figure 3). Bracts and bracteoles are small, at the base of the umbel. Purple flowers, with white to yellowish core, 10 to 15 mm in diameter, carried by a slender pedicel, 15 to 20 mm. The calyx is green, composed of 5 free, smooth, uneven sepals with orange or purple granulations. Corolla with 5 free petals, formed from a white claw and a pink purple lobe, spread on the widely rounded top. 10 hairy stamens, in 2 different sizes. The ovary is superior with 5 fused carpels. Fruit is rarely developed. It is an oblong capsule, 4 to 8 mm long, consisting of several seeds.

Table 1. Summary of the main morphological characteristics of mature plants of the *Oxalis* species

Species	Plant height (cm)	Underground structure	Leaf	Flower
Oxalis latifolia	20-30	Globular or ovoid bulb, reaching up to 2.5 cm long	Green leaflets, purplish beneath,	Pink/Purple flowers, with

		and 2 cm wide, surrounded by large papery protective scales, and brown feeder scales	entire, sub sessile, obtriangular or notched at the top, 1 to 5 cm long and 2 to 7 cm wide	white to yellowish core
Oxalis cornicu lata	5-20	Creeping and bending upwards, much branched, Softly to sparsely hairy, Stoloniferous	Green or purple three leaflets, alternate, wedge to heart shaped, deeply notched	Yellow flowers
Oxalis corymb osa	5-30	Bulbs globose to conic, scaly, bulbils many, clustered, globose to ovoid	Erect, three heart-shaped leaflets subequal, broadly obcordate, lobes rounded, orange-punctate on both surfaces especially near margin	Light reddish purple with darker nerves

Figure 2. Leaves of *Oxalis latifolia*

Figure 3. Flower of *Oxalis latifolia*

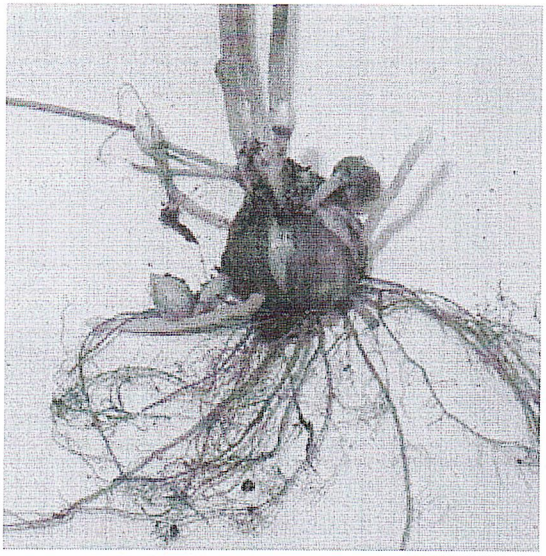

Figure 4. Bulb of the *Oxalis latifolia* with Bulbils

Figure 5. Growth habit of *Oxalis latifolia* in field (Source: CABI, 2021)

2.5.2 Identification of seed: Seed ellipsoid of 1 mm long, dark yellow to orange and ribbed.

2.6 Number of specimen sampled for identification

All the specific host fields should be monitored. At least 2-3 specimens in each field should be collected for identification.

2.7 Sampling methods

The weed sampling procedures can be followed as suggested by Colbach et al. (2000). They are described below and choose one of them, depending on field situations.

2.7.1 Methods based on random selection

There are four random methods:

a) **Random method:** It consists of choosing samples entirely randomly from the field and is often used in weed research.
b) **Distance10 method:** Samples are required to be separated by at least 10 m. The sampling procedure is as follows: the i^{th} sample is chosen randomly from the simulated field and its distance to each of the $(i\text{-}l)$ first samples is calculated; if any of these distances is smaller than 10 m, then the sample is discarded and a new one chosen; otherwise, the $(i+l)^{th}$ sample is selected.
c) **Distance20 method:** The procedure is the same as in the Distance10 method, but with a minimal sampling distance of 20 m.
d) **Stratified method:** The field is divided into five equal parts and then, a fifth of the required samples is selected randomly in each of these parts.

2.7.2 Methods based on systematic selection

The systematic positioning of samples is often used to ensure that samples are placed independently of the experimenter, avoiding or choosing unknowingly certain field areas while increasing sampled field area (Scherrer, 1983). There are two systematic selection methods:

a) **Diagonal method:** The samples are selected on the two diagonals of the field. The sampling process for $N=2n$ samples is as follows: the field (of length l and width w) is divided into n^2 rectangles of $l/n \times w/n$ m^2; the first sample is chosen randomly in the rectangle located on the field edge; if its coordinates are (x_1, y_1); then the coordinates of the i^{th} sample taken on the same diagonal are $[x_1+(i-l)\times w/n;\ y_1+(i-l)\times l/n]$ and the co-ordinates of the i^{th} sample taken on the second diagonal are $[x_1+(i-l)\times w/n;\ l-y_1-(i-l)\times l/n]$.

b) **Zig-zag method:** The samples are taken from three lines assembled vaguely as an "S". The sampling process for $N=3n-2$ samples consisted of dividing the field into $n \times N$ rectangles of $w/n \times l/N$ m^2. The first sample of co-ordinates (x_1, y_1) is again chosen randomly in the rectangle located on the field edge and the subsequent samples are chosen according to a protocol similar to that for the diagonal method.

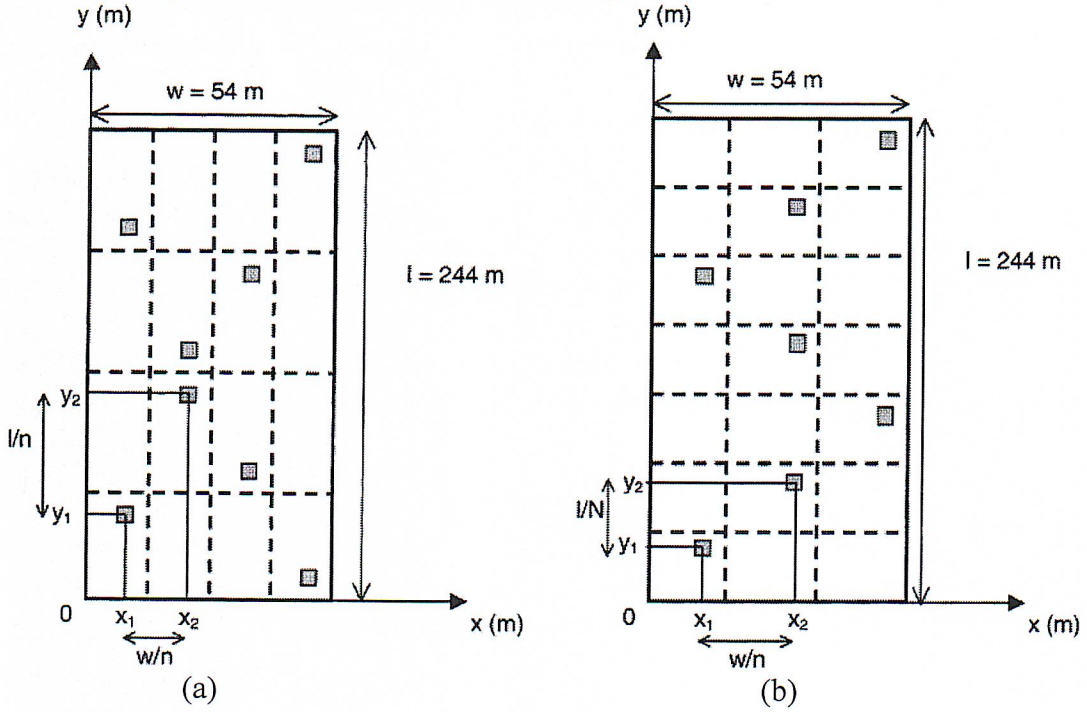

Figure 6. Example of a systematic sampling plan - (a) $n=4$ selecting eight samples ($N=2n=8$) and using diagonals, (b) $n=3$ selecting seven samples ($N=3n-2=7$) and using lines assembled as a zigzag (Colbach et al, 2000)

2.8 Specimen collection and preservation

2.8.1 Collecting technique

- Select normal looking specimens i.e. free of insect damage or disease.
- Collect plants that represent the range of variations in the population (2-3 plants in each field), not just a typical specimen.
- For mature plants, collect entire plant (leaves, bulb, roots) with flowers and/or seed heads
- Tag with collection number.

2.8.2 Take photographs of the specimen in the field

- Take pictures of the entire plant in its natural environment (remove any other vegetation around it).
- Make sure to capture leaves, stems, roots and flowers as much as possible.
- In many cases, one can remove the plant from the soil, brush or wash off the soil from the roots and lay it on a white paper background.
- Save the picture in a JPEG format with a clear resolution that allows one to see plant detail.

2.8.3 Precautions to be taken in the field

- Place the sample in a sealed plastic bag with moist paper towels (water may be sprinkled inside the bag, if needed) and keep cool or place it under a shade till transport.
- Press specimens in the field while collecting and whenever possible because delay causes a significant difference in the final shape and appearance (discoloration, structure etc.).

2.8.4 Pressing and drying

- Fold sufficient newsprint sheets (ca. 10) in half to form 45×30 cm folders and place plants to be pressed on the right half of the folder.
- Arrange plants carefully with a minimum overlap and flowers open showing both top and underside; leaves showing both upper and underside; bend or cut stem to accommodate the right half of the sheet.
- Turn the left half sheets over the specimens on the right half and press them using a plant press frame (45×30 cm) or by keeping under uniform weight covering the whole sheet area
- Loose seeds can be collected in separate packets and sealed.
- While pressing, prevent plant parts from curling or wrinkling during the drying process.
- Change the newspaper sheets in between, if they get moistened during drying. Drying can be done by placing the plant press frame with the specimens 15 cm above the home light bulb (60 or 100 watts) for ca. 24 hr

2.8.5 Mounting technique

- Keep ready herbarium sheet (mounting paper) (45×30 cm)
- Glue the label to the bottom right corner of the herbarium sheet.
- Run a bead of glue only along the top edge of the label.
- Arrange and glue the dried specimens on the herbarium sheet.
- Arrange the specimens on the sheet in such a manner that there will not be a balancing problem in storage.
- Attach the specimen to the mounting paper with thin ribbons of glue running from the paper across the plant part to the paper.
- A dot of glue beneath the flower head may be needed if the head is large and cannot be held down with a strap of glue on the petiole.
- If only one flower is placed on the sheet, protect it by gluing a transparent flexible covering over it.
- Use line straps or white gummed mounting tape to the size required to attach thick stems.

2.9 Data recording and mapping

- Data should be recorded in several aspects like
  - ✓ Date of collection
  - ✓ Collection number

- ✓ Locality
- ✓ GPS coordinates
- ✓ Elevation
- ✓ Host plant type and infestation severity
- ✓ Plant growth stage
- ✓ Local name(s)
- ✓ Habit and habitat

- Use mapping tools like GIS to create infestation distribution maps

2.10 Diagnostic laboratories

- National Agronomy Research Centre, Nepal Agricultural Research Council, Khumaltar, Lalitpur
- Central Agricultural Laboratory, Department of Agriculture, Hariharbhawan, Lalitpur
- Department of Agronomy, Agriculture and Forestry University, Rampur, Chitwan
- Department of Agronomy, Institute of Agriculture and Animal Science, Tribhuvan University, Kirtipur, Kathmandu
- Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu
- National Herbarium and Plant Laboratories, Department of Plant Resources, Godawari, Lalitpur
- Natural History Museum, Swayambhu, Kathmandu

3 Reporting

The responsible or concerned organizations (diagnostic laboratory) or an independent surveyor, after analysis and identification, should submit a report to the NPPO-Nepal for the reporting/declaration of weed. The reports should include infestation maps, photographs and specimen vouchers. If specimens cannot be identified morphologically, they should be identified by molecular methods.

4 Record keeping

NPPO-Nepal, in collaboration with responsible laboratories, will preserve the specimens and keep all the records safely. The documentation system should be well maintained by the NPPO-Nepal and the collaborating institutions will have access to it.

5 Molecular diagnosis of *Oxalis latifolia*

A number of methods are available for molecular diagnosis of plant species. They involve different steps, starting from genomic DNA extraction to their sequencing. One of the most commonly used methods is described below. However, the method is not necessarily mandatory to follow. Any other established/adopted methods may be used alternatively.

5.1 Sample preparation and genomic DNA extraction by CTAB method

The CTAB DNA extraction method is cheap, effective and applicable for a wide range of applications including DNA barcoding, shotgun sequencing and log-read sequences. The protocol utilizes organic solvents and the harmful nature of some of the solvents along with relatively long time period consumption to complete the protocol can, therefore, be a limitation of using this method.

The CTAB method given by Doyle and Doyle (1990) was modified by Tiwari et al. (2012), and it can be used for genomic DNA extraction from plants.

- Take 1 g of plant parts (leaves, nodal region and meristematic region) each separately from the selected plants.
- Wash properly and air dry them to remove any trace of water present.
- Add 2 mL of CTAB extraction buffer to them, and grind them properly using mortar and pestle.
- Transfer the paste to Eppendorf tubes and keep them in a water bath at 65°C for 45 min.
- Take the tubes out of the water bath and keep at room temperature for 5 min, and then centrifuge it at 12,000 rpm for 15 min.
- Take 1 mL supernatant from each tube, transfer them to another tube with a wide bore pipette, add 500 µL of Phenol (P):Chloroform (C):Isoamyl alcohol (I) to each tube, shake for 15 min in a shaker, and centrifuge at 12,000 rpm for 12 min.
- Take supernatant again in another Eppendorf tube, add equal volume of P:C:I, shake well, and centrifuge at 12,000 rpm for 10 min.
- Transfer supernatant to a new Eppendorf tube, add equal volume of C:I, shake well, and centrifuge at 10,000 rpm for 10 min.
- Transfer the supernatant (containing DNA) to a new Eppendorf tube and add pre-chilled isopropanol (2/3 amount of the supernatant), keep at -20°C for 2 hr, then centrifuge at 8,000 rpm for 8 min.
- Discard the supernatant from tubes, add 300 µL 70% ethanol to the pellet, mix well, centrifuge at 8,000 rpm for 10 min; discard the ethanol, dry the pellet by incubating it at 37°C in a dry bath for ca. 1 min, dissolve the pellet in 100 µL of TAE buffer and store at -20°C in a deep freezer.

5.2 Quantification of extracted genomic DNA

By using a spectrophotometer (like NanoDrop 1000, Thermo-Fisher, USA), quantitative analysis of DNA (ng/µL) can be assessed through comparison of the absorption ratio for A260/A280. The reagent contamination can also be assessed using the NanoDrop by comparing the absorption ratio of A260/A230. DNA integrity and fragment size can be assessed qualitatively using 1.5% agarose gel electrophoresis stained with ethidium bromide.

5.3 Polymerase chain reaction and sequencing

The marker that demonstrates sequence variations that offer sufficient discriminatory power to distinguish closely related species is ideal for species identification in a diverse family. The Consortium for Barcode of life (CBOL) has established various working groups dedicated to identifying universal barcode genes for different taxonomic groups, including cytochrome oxidase subunit I (*COI*) in metazoans, maturase K (*matK*), ribulose-1,5-bisphosphate carboxylase (*rbcL*), internal transcribed spacer (*ITS*) in plants (Antil et al., 2023). These primers produced specific amplicon sizes (i.e., 507bp of *ITS1*; 550bp of *rbcL* & 850bp of *matK*) while running PCR reactions consisting of approximately 50 ng per 1 µL of template DNA. The amplified products can then be sent to DNA sequencing laboratories (like Apical Scientific Sdn. Bhd., Malaysia) for bidirectional sequencing. Sequencing can be performed using the same primers employed for amplification through capillary electrophoresis on the Sanger DNA Sequencer. The resultant sequences after editing using the software like BioEdit V7.0.9.0 and publicly available sequences in NCBI portal

for the *matK* gene, *rbcL* gene and *ITS* gene should be included in the analysis procedure using different methods for detection of species.

Table 2. List of potential primers that can be used for PCR and their sequences

Region	Primer	Sequence 5'-3'	Tm (°C)	References
ITS1	5a fwd	CCTTATCATTTAGAGGAAGGAG	50	Chen et al., 2010
	4 rev	TCCTCCGCTTATTGATATGC		
ITS2	S2F	ATGCGATACTTGGTGTGAAT	56	Chen et al., 2010
	S3R	GACGCTTCTCCAGACTACAAT		
matK	3FKIM	CGTACAGTACTTTTGTGTTTACGAG	52	Costion et al., 2011
	1RKIM	ACCCAGTCCATCTGGAAATCTTGGTTC		
rbcL	rbcLa-F	ATGTCACCACAAACAGAGACTAAAGC	62	Kress et al., 2009
	rbcLa-R	GTAAAATCAAGTCCACCRCG		

Table 3. PCR reaction condition for ITS region (Chen et al., 2010)

Reagent	Volume (µL)	Final concentration
PCR master mix	12.5	1X
Forward primer (10 µM)	1.25	0.5 µM
Reverse primer (10 µM)	1.25	0.5 µM
Diluted template DNA	1.0	50 ng/µL
Nuclease free water	9.0	n/a
Total volume	25	

Table 4. Steps for PCR amplification of ITS region (Chen et al., 2010)

Stages	Temperature	Time	Size range (bp)
Initial denaturation	94°C	5 min	707 bp for ITS1
Final denaturation	94°C	1 min	571-1153 bp for ITS2
Annealing	50°C	1 min	
Elongation	72°C	1.5 min	
Final extension	72°C	7 min	
Holding	4°C	∞	

5.4 Electrophoresis

PCR products are subjected to an agarose gel (1.5-2%) electrophoresis.

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Detection survey protocol for *Oxalis latifolia* Kunth.

NPPO-Nepal, 2025

Appendix 1. Sample of a page of field book

Flora of ..... State.....

Serial ..... Date.....

Name.....

Family.....

Local Name.....

Habit.....Height.....Fl..... Fr.....

Locality..... Alt.....

Soil.....

Vegetation type.....

Associated plants.....

Distribution..... Abundance.....

Uses.....

Significant notes.....

.....

.....

.....

Photograph..... Collector.....

Identified by.....

Appendix 2. Label attached on herbarium sheet

HERBARIUM	
11	aa
Serial no. : bb	Family: cc
Latin name dd	Common name ee
Local name ff	Location gg
Date of collection hh	Collected by ii
Identified by	Verified by
Remarks:	

Appendix 3. Mounting plant specimen in a herbarium sheet