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Morphological and molecular identification and management of chafer beetle (*Protaetia terrosa* Gory & Percheron) infesting cluster bean in Rajasthan

Shalini Pandey[1]*, M M Sundria[1], Kolla Sreedevi[2], Ankur Tripathi[3], Veena N V[2] & Ram Singh[4]

[1]Agricultural Research Station, Agriculture University, Mandor 342304, Jodhpur, India

[2]ICAR-National Bureau of Agricultural Insect Resources, Bengaluru 560024, Karnataka, India

[3]College of Dairy and Food Technology, Agriculture University, Mandor 342304, Jodhpur, India

[4]Chaudhary Charan Singh Haryana Agricultural University, Hisar 125001, Haryana, India

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Identification, incidence, and management of the chafer beetle, *Protaetia terrosa*, on cluster beans was carried out in semi-arid Indian conditions. The *P. terrosa* was identified using morphological and molecular characters. The distinguishing morphometric characteristics of *P. terrosa* were *viz.*, head (length: 2.21 mm; width: 2.94 mm), thorax (length: 4.95 mm; width: 6.87 mm), elytra (length: 10.08 mm; width: 8.68 mm), and other morphological features. For molecular identification, a gene fragment of 655 bp size encoding mitochondrial cytochrome c oxidase I enzyme was amplified, sequenced and submitted to NCBI (MW008478), and the barcode was generated as BIN Number BOLD: AEF1461. This is the first report of the partial mt Co1 gene sequence of *P. terrosa*, with a unique barcode as diagnostic tool. The BLAST P and phylogenetic analysis revealed highest sequence similarity of *P. terrosa* with *P. cuprea*, and *P. fusca*. The *P. terrosa* infestation results in wilting, drying and ultimately dying of cluster bean plants. The chafer beetle infested plants have a visible white portion (pith) of the stem with little or devoid of lateral roots. The study recorded up to 14.23% infestation by chafer beetles under natural unprotected conditions. The soil drenching with clothianidin 50% WDG @ 250 gm/ha resulted significant reduction in damage by *P. terrosa*, and could prevent up to 19.36% loss in yield. In nutshell, regular crop monitoring and adoption of suitable management practices are highly important to keep this pest under check.

Keywords: *Cyamopsis tetragonoloba*, *Protaetia terrosa*, Identification, Morphological features, Molecular approach, Management

Cluster bean (*Cyamopsis tetragonoloba* L.) is one of the multipurpose underexplored crops grown for food and fodder purposes in loamy and sandy soils of arid and semi-arid regions[1-5]. It is a drought-adapted annual legume having comparatively low water requirements than other dry land legumes[3]. India is the main producer of cluster bean, contributing about 90% to the global production[6]. In India, the crop is mainly grown in Rajasthan, Haryana, Gujarat, Punjab and Uttar Pradesh states under rainfed conditions[6]. Rajasthan state accounts for about 87.7% of the production and 91.5% of the acreage during 2020–21[6,7]. This nutrient-dense legume is sometimes termed a functional food for having high protein and carbohydrate content and appreciable amount of micronutrients (Vitamins and Minerals), unsaturated fatty acids, and bioactive components with nutraceutical properties[8]. Cluster bean seed contains about 30 to 33% gum in endosperm therefore has gained the popularity as an industrial crop[2]. Of the total cluster bean production in India, 90% is exported for shale gas and oil industries[9]. It has excellent yielding potential however, the attack of a magnitude of insect-pests[10, 11] during different growth stages cause significant reduction in yield. Whitefly, *Bemisia tabaci* (Genn.), aphid, *Aphis craccivora* Koch; Pod borer, *Helicoverpa armigera* (Hub.), *Acaudaleyrodes rachipora* (Singh), *Maruca testulalis* Geyer; leaf hopper, *Empoasca motti* Pruthi; leaf perforator, *Dichomeris inthes* Meyr are important regular insect pests of cluster bean[12, 13] but when *P. terrosa* appears on the crop it was found to be the most destructive insect causing mortality of plants. Herein, we report the identification and management of Chafer Beetle, *P. terrosa* infesting cluster bean in semi-arid conditions of Rajasthan, India. We used morphological and molecular tools for identification of the chafer beetle.

Materials and Methods

The field experiment was conducted on cluster bean variety *viz.*, RGM-112 to identify the insect pests, their nature and extent of damage at Agricultural Research Station, Mandor, Rajasthan, India. Experimental plot size was 3.1 × 4.0 m^2 and plant spaced at 30 cm row to row and 10 cm plant to plant. Five plants from each of three plots were observed randomly to record the presence or absence

*Correspondence:
E-mail: pandeyshalini80@gmail.com

of insects. During the studies, severely damaged and wilted plants were examined carefully for the incidence of insects. The damaged plants were found affected by the chafer beetles in the root zone. The beetles were collected in the polythene bags and brought to Entomology laboratory. The specimens were then transferred to vials containing 90% alcohol and five specimens were observed for morphological and molecular characterization.

Morphological characteristics

The diagnostic characters of female beetle specimens were observed using Leica M60 Stereozoom microscope and the beetles were identified up to species level using taxonomic keys[14].

Molecular characterization

The specimens used for morphological identification were also used for molecular characterization and remaining specimens were kept as voucher specimens as dry collection and alcohol preserved specimens by transferring to 95% ethanol and stored at 20°C[15].

DNA Extraction, PCR amplification and phylogenetic analysis of mitochondrial cytochrome oxidase subunit 1 (Mt CO 1) gene

The DNA extraction of mitochondrial Co 1 region was done from the leg portion of the insect using QiagenDNeasy® kit by following kit's standard procedure and protocol. The DNA template was subjected to PCR amplification of a 658 bp region near the 5' terminus of the *cox1* gene following standard protocol[16]. Primers used were: forward primer (LCO 1490: 5'-GGTCAACAAATCATAAAGATATTGG-3'), and reverse primer (HCO 2198: 5'-TAAACTTCA GGGTGACCAAAAAATCA-3'). PCR reactions were carried out in 96-well plates, 50 µL reaction volume containing: 5 µL GeNeiTMTaq buffer, 3 µL GeNeiTM 10mM dNTP mix, 1 µL (20 pmol/µL) forward primer, 1 µL (20 pmol/µL) reverse primer, 1 µL GeNeiTMTaq DNA polymerase (1 U/µL), 8 µL DNA (50 ng/µL), and 31 µL sterile water. Thermo cycling consisted of an initial denaturation of 94°C for 5 mins, followed by 34 cycles of denaturation at 94°C for 1 min, annealing at 45°C for 30 sec, extension at 72 °C for 1 min and final hold at 4°C. PCR was performed using BioRad C1000™ Thermal Cycler. The amplified products were analysed on a 1.5% agarose gel electrophoresis[17]. Analysis of the gel was performed by Gene Sys software. The amplified products were sequenced by M/s Barcode Bioscience, Bengaluru. Each specimen PCR sample was bi-directionally sequenced and checked using NCBI-BLAST. The Co 1 generated sequences have been deposited in NCBI Genebank database. Subsequently, barcode was also developed (Fig. 1). The Mt CO 1 protein sequences were downloaded from NCBI (http://www.ncbi.nlm.nih.gov/protein/) and aligned using Clustal W algorithm (http://www.ebi.ac.uk/Tools/msa/clustalw2/)[18]. The aligned sequences were used to build phylogenetic tree using Mega X software[19]. The phylogenetic tree was built using the following parameters: bootstrap method with 1000 replications, number of differences as the substitution model, and complete deletion for gaps/missing data treatment.

Infestation and Management of Chafer beetle

The counts of beetles associated with the infested plants were recorded weekly from the first week of August to end of September in *kharif* season of the year 2018 and 2019. Observations on plant damage by the beetle were also recorded by counting total number of damaged plants to healthy plants in each plot and further plant damage (%) was calculated. During the experimental year 2018, beetle population was high and caused significant yield losses in the crop (Fig. 2), therefore its management through insecticide was planned during the experimental year 2019.The experiment was laid out in two sets *viz.*, protected with insecticide and unprotected crop plots. In the protected treatment, crop was drenched with

Fig. 1 — *Protaetia terrosa* barcode

Fig. 2 — Cluster bean plants showing infestation of *Protaetia terrosa* beetles

clothianidin 50% WDG @ 250 gm/ha after onset of the beetle infestation. Loss assessment was observed by calculating avoidable loss (%) in yield by comparing the yield from protected plot treated with clothianidin 50% WDG @ 250 gm/ha with unprotected (untreated) plot using the following formula given by LeClerg[20].

$$\text{Avoidable yield loss (\%)} = \frac{\text{Yield of protected plot} - \text{Yield of unprotected plot}}{\text{Yield of protected plot}} \times 100$$

Plant infested by beetles and yield obtained from protected and unprotected plots analyzed by paired't' test[21].

Results and Discussion

In this study, beetles associated with the wilting of the cluster bean plants were identified as *Protaetia terrosa* (Gory and Percheron 1833) belonging to tribe Cetoniini, subfamily Cetoniinae of Scarabaeidae based on the taxonomic keys. It was identified as a new emerging insect pest of cluster bean grown under semi-arid conditions of Rajasthan, India during June-July cropping season. The beetles were identified as *P. terrosa* based on the taxonomic keys[14].

Morphological characterization of *Protaetia terrosa*

There are no recent descriptions of *Protaetia terrosa* and hence its various morphological characters are herewith furnished. Body medium sized, oval, convex, black, with green hue, upper surface covered with an opaque bloom, lateral sides of the pronotum, elytra, pygidium and sternum decorated with irregular grey patches with one row of white patches on each side of the abdomen (Fig. 3A & 3B).

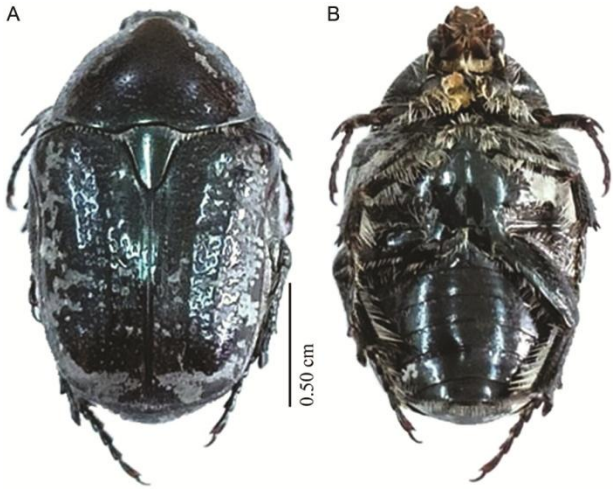

Fig. 3 — *Protaetia terrosa* adult beetle a. dorsal view (A), *Protaetia terrosa* adult beetle b. ventral view (B)

Head rugose, clypeus not elongate, broad in front with clypeal margin reflexed, armed with two sharp short teeth, Mandibles are thin, not sharp-pointed and furnished with a free membranous inner lobe, eyes are prominent, antennae with three lamellate club. The labrum membranous, not visible externally, mandibles not visible externally, front coxae vertical. Pronotum strongly and evenly punctured with sides rounded, abruptly emarginated behind and deeply excised before scutellum not forming a transverse line, base of the pronotum not meeting the ridges upon the elytra and scutellum. Mesonotum transverse before the coxae and mesosternal process broad, flat and setose with yellow hairs. Scutellum elongated, broad in front with few punctations in the anterior angles. Elytra metallic, sinuated behind the shoulders, coarsely punctured in rows with apical angles not produced, mesosternal epimera reaching the dorsal surface and dilated, metasternum rugose, abdomen with visible six segments ventrally and smooth, elytra do not cover fully, hind coxa and abdomen partially visible from above, pygidium rugose, front tibiae strongly tridentate, all tarsi short and not spinose. Morphometric dimensions of *P. terrosa* beetle are head length: 2.21 mm and width: 2.94 mm; thorax length: 4.95 mm and width: 6.87 mm; elytral length: 10.08 mm and width: 8.68 mm.

Molecular characterization

The species subjected to molecular characterization yielded *mt Co 1* gene fragment of 655 bp size, and the sequence of gene fragment was submitted to NCBI (MW008478). Subsequently the barcode was also generated with BIN Number BOLD: AEF1461. This is the first report of the partial *mt Co1* gene sequence of *P. terrosa*. The voucher specimens of adult beetles are deposited at National repository of ICAR-National Bureau of Agricultural Insect Resources (NBAIR) Bengaluru. The amplified gene fragment sequence was subjected to BLAST P and phylogenetic analysis and the results revealed highest sequence similarity of *P. terrosa* with *Protaetia cuprea*, and *Protaetia fusca* (Fig. 4). Though *P. terrosa* phylogenetically clustered with the above species but they are morphologically different from each other. *P. terrosa* was found distantly related to *P. aurichalcea* and *P. alboguttata* that might be possible due to adaptation to different environmental condition and their varied distribution. Though we have identified *P. terrosa* beetle infesting cluster bean plants from Western Rajasthan, further investigation

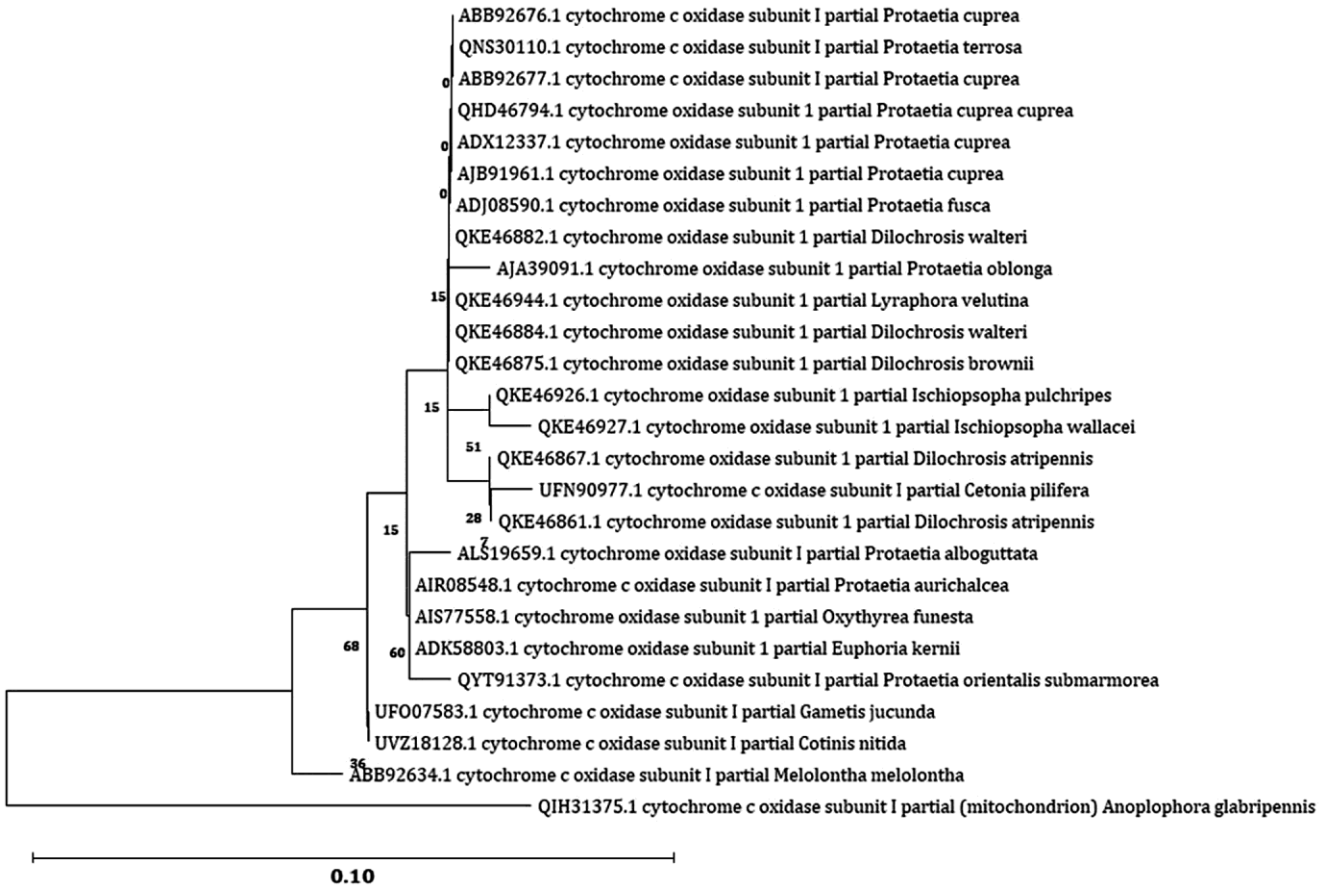

Fig. 4 — Phylogenetic analysis of *Protaetia terrosa* with other *Protaetia sp.,* some members of Scarabaeidae family and *Anoplophora glabripennis (Family-Cerambycidae*. (QNS30110.1-*Protaetia terrosa*; ABB92676.1, ABB92677.1, QHD46794.1, ADX12337.1& AJB91961.1- *Protaetia cuprea*; ADJ08590.1- *Protaetia fusca*; QKE46882.1&QKE46884.1- *Dilochrosis walteri;* AJA39091.1-*Protaetia oblonga;* QKE46944.1- *Lyraphora velutina;* QKE46875.1-*Dilochrosis brownii*; QKE46926.1- *Ischiopsopha pulchripes;* QKE46867.1 & QKE46861.1- *Dilochrosis atripennis;* UFN90977.1- *Cetoniapilifera*; ALS19659.1- *Protaetia alboguttata*; AIR08548.1- *Protaetia aurichalcea*; AIS77558.1- *Oxythyrea funesta*; ADK58803.1- *Euphoria kernii*; QYT91373.1- *Protaetia orientalis* submarmorea; UFO07583.1- *Gametis jucunda*; UVZ18128.1- *Cotinis nitida*; ABB92634.1- *Melolontha melolontha*; QIH31375.1; *Anoplophora glabripennis*

is still needed to assess the relationship of *P. terrosa* with other *Protaetia* species as this genus is highly polymorphic and widely distributed. Research is in progress for further molecular analysis of *P. terrosa* using different reference genes.

Various reports have been published documenting its distribution from Gujarat, Madhya Pradesh, Maharashtra, West Bengal, Karnataka and Rajasthan states in India[14, 22, 23,24]. In Rajasthan state, *P. terrosa* was reported infesting cluster bean crop in Jodhpur district[22] in the year of 1998 and thereafter from Gujarat state during the year1999[23] as a new emerging pest of cluster bean. After the year 1999, pest was not reported to cause considerable and frequent economic damage in India. However, after a span of 20 years, *P. terrosa* was found infesting cluster bean in serious proportions in Jodhpur region of Rajasthan. The related species of *P. terrosa* namely *P. alboguttata* (Vigors), *P. aurichalcea* (F.) and *P. maculata* (F.) have been found damaging maize (*Zea mays* L.), sorghum (*Sorghum bicolour* L.) and bajra crops (*Pennisetum typhoides* S. & H.) in the state of Rajasthan[25]. Tandon *et al*.[26] reported *P. cinerea* as a damaging pest on brinjal (*Solanumm elongena* L.) from Bangalore, Karnataka. Similarly, *P. alboguttata* infesting maize tassels, *P. cinerea* infestation on rose and brinjal have also been reported from Bangalore, Karnataka[27, 28]. Kamala Jayanthi *et al*.[29] reported that *P. alboguttata* damaged ripe karonda, *Carissa carandas* fruits (22.40 ± 2.50%) in Bangalore, Karnataka. Other species of genus *Protaetia viz., P. impavida* (Janson) and *P. coenosa* (Westw.) from Western Himalaya; *P. andamanarum* from Andaman; *P. bidentipes* from Nicobar; *P. fusca* from Andaman,

West Bengal and Assam; *P. acuminata* from Andaman and Nicobar; *P. cariana* from North India [29, 30, 31] have been reported to cause partial damage to different host plants.

Infestation and Management

Initial symptoms of beetle infestation appeared in the form of drooping of leaves and partial wilting of the cluster bean plants (Fig. 5A). Upon digging the root zone of the damaged plant, beetles were present in soil upto 5-10 cm deep (Fig. 5B). Damage was apparent on subsoil portion of stem as well as to the roots which lead to the disruption of uptake of water and other nutrients from the soil, thereby leading to gradual wilting of plant. As a result of feeding on the stem portion of the plant by the beetles, the internal white portion (pith) of the stem was distinctly visible. The root portion also exhibited clear damage symptoms. The damaged plant had little or no lateral roots and uprooted easily from the sandy soil (Fig. 5C).

Fig. 5 — Wilting of the plant due to infestation of *Protaetia terrosa* beetle (A); *Protaetia terrosa* beetle near the subsoil portion of the plant (B); Damaged subsoil portion of the plant due to *Protaetia terrosa* beetle (C)

During both experimental years (2018 and 2019), the incidence of beetle was appeared about 30 days after germination and remained up to the onset of pod formation. Beetle infestation appeared from first fortnight of August and beetle population reached maximum in second fortnight of August and then started declining in the month of September and reached lowest in the second fortnight of September. Highest population of the beetles (2.20 and 3.00 beetles/plant) was observed in the fourth week of August month of both the experimental year, respectively (Fig. 6).

During the year 2018, the plants infestation by beetles was 14.23% without any protection under natural conditions. During 2019, before application of insecticide, there was no significant difference (P<0.05) in plants damaged by beetles in protected (13.27%) and unprotected (12.26%) plots. After drenching with clothianidin insecticide, there was a significant difference ($P \leq 0.05$) in plants damaged by beetles between protected (0.32%) and unprotected (12.33%) plots (Table 1). Yield of cluster bean in protected and unprotected treatments was 1312 kg/ha and 1058 kg/ha, respectively (Table 1). Avoidable yield loss was 19.36% after drenching with clothianidin 50% WDG @ 250 gm/ha. *P. terrosa*

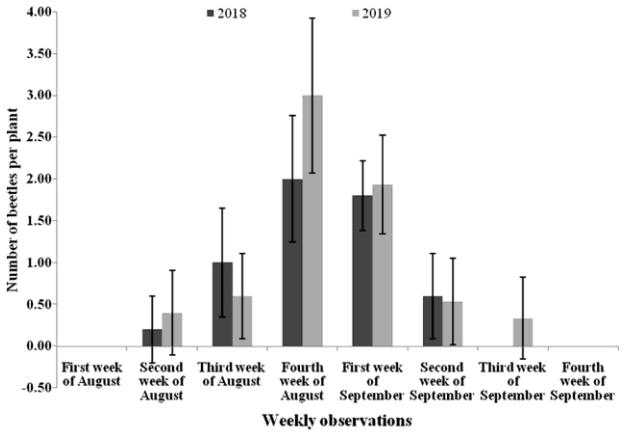

Fig. 6 — Number of *Protaetia terrosa* beetles per plant on infested surviving plants of clusterbean during the year 2018 and 2019

Table 1 — Comparative plant damage by Chafer beetle, *Protaetia terrosa* and yield under protected and unprotected conditions during the year 2019

Treatment	Percent plant damaged		Yield (kg/ha)	Avoidable loss (%)
	Before treatment	After treatment		
Protected-Drenching with Clothianidin 50% WDG @ 250 gm/ha	13.27	0.32	1312	19.36
Unprotected	12.26	12.33	1058	-
t calculated	0.97	79.03	5.70	-
[*t tabulated = 2.18 at 12 d.f. and 5% level of significance]				

caused extensive damage to roots of cluster bean with 28.66% plant mortality and 4.40 beetles/plant, has been reported from Jodhpur region of Rajasthan[22].

Only adult beetles of *P. terrosa* causes damage to the crop[23]. The adult beetle burrows the soil, enter the root zone and damage the root by gnawing the outer fleshy skin portion which leads to death of the plant. They further observed large number of grubs and earthen cocoons of this beetle on the bunds under *Azadirachta indica* trees. *P. terrosa* activity starts immediately after the onset of monsoon (July) and its peak activity observed from 2nd to 3rd week of August[23]. By the onset of monsoon, the adults emerge out and lay the eggs in the debris. When the favourable conditions (monsoon rains) appears, beetle activity increases, and beetle population starts to build up by the 2nd and 3rd week of August. The population peak appears in the 4th week of the August and thereafter decreases. Beetle is a univoltine species (one generation per year) and spends most of its life in the debris and decaying matter[23]. It remains active in the field before the crop becomes unsuitable for consumption and pest growth (Second fortnight of September).

Conclusion

The Chafer beetle, *Protaetia terrosa* (Coleoptera: Scarabaeidae: Cetoniinae) was observed infesting the cluster bean crop in Mandor region, Jodhpur, Rajasthan, India. The infestation was observed in severe form during the year 2018 and 2019. The chemical treatment with clothianidin 50% WDG @ 250 gm/ha caused significant reduction in beetle damage attaining an avoidable yield loss of 19.36%. Morphological attributes and *mitochondrial Co 1* gene sequencing of this species, *P. terrosa* were also attempted for the first time and the sequence of 655bp has been deposited in the NCBI and BOLD databases to secure its identity. The study concluded that regular monitoring of cluster bean crop and adoption of suitable management practices are required to keep this pest under check.

Conflict of interest

The authors declare no conflict of interest.

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