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Bionomics and biorational management of Singhara beetle, *Galerucella birmanica* Jacoby, a potential threat to water chestnut production in India

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Abstract
Water chestnut or '*Singhara'* or '*Paniphal'*, is an important annual aquatic vegetable in many parts of India. The plant was severely infested by Singhara beetle, *Galerucella birmanica* during July to November in Varanasi region. Almost 100% plants and 70 – 80% leaves were infested by the grubs and adults of this beetle. Under laboratory conditions the biology of the pest revealed that, the incubation, larval and pupal periods were 4.15 ± 0.56, 12.75 ± 0.80 and 2.80 ± 0.40 days, respectively. Adult females lived longer (21.10 ± 1.57 days) than the males (16.38 ± 0.77). Gravid females laid on an average 90.4 ± 14.67 eggs, whereas egg viability was 58.9 ± 3.75 per cent. Amongst the different biopesticides tested, neem oil was found most promising having lowest median lethal time of 17.32 h followed by *Metarhizium anisopliae* IIVR strain (52.67 h). Combinations of these entomopathogenic fungi (EPF) and neem oil (1:1) had lower LT_{50} values than each of their individual indicating the compatibility among them. The present study will be helpful in developing future integrated management strategies of this nefarious pest in India relevant to aquatic cropping systems.

Keywords: *Galerucella birmanica*, water chestnut, biology, seasonal incidence, biopesticides

1. Introduction
Water chestnut (*Trapa natans* Linn.), belonging to the family Trapaceae and commonly known as 'Singhara' or 'Paniphal' in India, is an annual, aquatic plant found in stagnant water in ponds, lakes, wetlands and tanks. It occurs all over India, Pakistan, Ceylon, South Eastern Asia, Malaya, tropical Africa [1] and Burma [2]. In India, this aquatic vegetable is mainly cultivated in the states of Madhya Pradesh, Uttar Pradesh, Bihar, Orissa, Assam, West Bengal and Tripura where high rainfall is conducive for its successful cultivation. It is grown mainly for human consumption either in the form of vegetable, dried to make flour to prepare flattened bread *i.e.*, chapatti or in the shape of sweet dishes of many kinds according to individual's taste [3]. The fruits of the water chestnut are crunchy in texture and sweet mild flavoured, with remarkable nutritional properties. Their medicinal properties have rendered them usable in Ayurvedic and Unani systems of medicines [4].

Insect pests and diseases are the major biotic constraints for vegetable production. The losses in vegetables crops in the country due to various insect pests range on an average from 10-30 per cent [5, 6]. The crop water chestnut is also attacked by several insect pests throughout its growth period [7]. Amongst them the water chestnut beetle, popularly known as Singhara beetle, *Galerucella birmanica* Jacoby (Chrysomelidae: Coleoptera) is of much significance to this crop in India and China [8,9]. Due to its potential to cause serious damage on water chestnut, this deadest pest is recommended as a promising biocontrol agent of *Trapa natans*, considered as an obnoxious weed, in countries like China.

To control this nefarious pest, Indian farmers are commonly used many synthetic insecticides which may lead to problems like resistance to insecticides, resurgence of target insects, and secondary pest outbreak, in addition to these, residues to food and beverages, contamination of groundwater, adverse effect on human health and wide spread killing of non-target organisms [10, 11, 12]. This is the insect from which first insecticides resistance against DDT and BHC [13]. Considering the ill-effects of these chemical insecticides, presently the major emphasis is being given on biocontrol approaches in the present context of environmental safety. Biological control of insect pests using different entomopathogenic microorganisms are gaining importance due to their target specificity, self-perpetuity and obvious safety to the environment [14, 15]. Therefore, an attempt was made to study its biology, seasonal incidence and its biorational management. The present study will help to identify the most vulnerable stage of the pest and suitable biocontrol and biopesticides(s) for its timely control.

2. Materials and Methods
All the experiments were conducted at ICAR-Indian Institute of Vegetable Research (83º53´E longitude and 18º52´N latitude), Varanasi, Uttar Pradesh, India at 28 ± 2ºC temperature, 70–80% relative humidity and a photoperiod of 13:11 (L:D) h.

2.1 Seasonal incidence
The regular monitoring at weekly intervals for *G. birmanica* was recorded during morning (in between 10 to 11 am). The population of the beetles was counted from ten randomly selected plants and expressed as number of beetle population per water chestnut plant. The data were recorded from January to December.

2.2 Biology
To study the biology of the pest, fresh uninfested water

chestnut plants were collected from the ponds maintained at research farm of ICAR-Indian Institute of Vegetable Research, Varanasi and placed in partially water filled plastic jar (diameter 11 cm and height 13.9 cm) in such a manner that root portion of the each plant was submerged in water and foliar part was outside the jar. Each plastic jar was again placed in a wooden cage (38 x 34 x 42 cm) and kept in biocontrol laboratory. Some of these sets (twenty numbers) were used for maintenance of stock culture of beetle from the eggs laid by initially collected from the ponds. Newly emerged (24 h old) *G. birmanica* adult male and female (2:1 ratio) from stock culture were released into the cage and observations on various biological parameters were made at 8 h intervals. Gravid females were allowed to oviposit on the leaves till their death. Eggs, laid in small batches, were counted using a hand lens. After egg laying, only 5-7 eggs were kept in each plant for further study and excess eggs were removed by hair brush. Thirty replications were maintained for recording data on different biological parameters. Incubation period was recorded after hatching and each neonate grubs were distributed on different leaflets of the same plant. Duration of different instars were recorded by observing the moulting and expressed in days. When the larvae reached to the pupal stage, they were collected individually and placed into another plastic petri plate (9.8 cm dia and 9.4 cm depth). The pupae were observed daily until adult emergence.

2.3 Bioassays

Talc based formulations of promising entomopathogens *viz*., *Beauveria bassiana* IIVR strain (1 x 10^{10} cfu/ g), *Metarhizium anisopliae* IIVR strain (1 x 10^{10} cfu/ g) and *Lecanicillium* (= *Verticillium*) *lecanii* commercial strain (1 x 10^{8} cfu/g) were taken for the experiments. Neem oil (1%) was prepared by dissolving in emulsifying water containing Triton X-100 (0.5 g in 100 ml of distilled water) as an emulsifier. All the entomopathogenic fungi were prepared at their recommended doses (@ 5 g/l of water) under laboratory conditions. Ten newly emerged adults *G. birmanica* grubs were placed in each petri dish (9 cm dia x 1.8 cm depth) and directly sprayed with 1 ml of each concentration of different microbial insecticides alone and their combination with neem oil (1:1) under Potter's tower at 340 g/cm^2 pressure. The sprayed petri dishes containing the treated test insects were dried for 5 min under fan. The water chestnut leaflets of the size 6 x 3.5 cm, wrapped with cotton moistened with distilled water, were used as food material for the adults during post-spray period [16, 17]. Ten adult insects comprised a replication and five replications were maintained for each treatment. For assessment of toxic effect, mortality counts were taken at 8 h interval each till 5 days after application of respective the treatments and moribund insects were considered as dead.

2.4 Data analysis

The mortality data were corrected by Abbott's formula [18] and Probit analysis [19] was done with SAS program (version 9.3) for calculating LT_{50}. Any two values of median lethal times (LT_{50}) determined were considered significantly different if their respective 90% confidence limits (CL) did not overlap.

3. Results and Discussion

3.1 Seasonal incidence

The incidence of *G. birmanica* on water chestnut started from July onwards and continued till first fortnight of December with a peak during October when 100% plants were severely affected by this beetle. From second fortnight of December onwards coinciding with the intense winter in the region, beetle gradually disappeared. However, a few adults were noticed to hide in soil cracks and crevices around the ponds during the end of December. Thereafter the population was practically nil. It again started from July and start breeding. *G. birmanica* occurred on water chestnut during April to December in Uttar Pradesh [20].

3.2 Biology

From the table 1 it is evident that Singhara beetle, *G. birmanica* had life-cycle of 33 – 46 days under laboratory conditions. Gravid females laid eggs on upper surface of the leaves in small batches of 5-13 eggs and in her life-time laid up to 111 eggs with an average of 90.4 ± 14.67 eggs. They deposited the eggs mostly during night time and sometimes during day also. Egg viability ranged from 53 – 64 per cent. The freshly laid eggs were small, round in shape, light yellow in colour and gradually turned to reddish brown in colour before hatching. The incubation period varied from 3.5 to 5.5 days with an average of 4.15 ± 0.56 days. The neonate grubs were light brown in colour and gradually turned to dark brown with age. The grubs were cruciform, stout and elongate with a well-developed sclerotized head. Immediately after hatching, they remain passive for a while and then gradually move to different directions on the leaf and started biting on the upper epidermis of the leaf. Larvae passed through three instars to become pupa. The first, second and third instar larval period ranged from 3.25 to 4.25, 4.5 to 5.25 and 4 to 4.75 days, respectively. Each instar had a prominent eucephalous head with three pairs of thoracic legs and tapering body. Duration of the total larval period ranged from 11.75 to 14 days. Critical observation revealed that during pupation the third instar full grown larvae stopped its feeding and became sedentary by settling itself on leaf surface through a gummy secretion from its anus. The pre-pupal stage lasted for 2.5 – 3.5 days. The pupa was exarate, bright orange yellow in colour and measuring about 3.79 to 5.12 mm in length and 3.34 to 3.61 mm in width across the thorax. The pupal period ranged from 3.50 to 4.75 days with an average of 3.98 days ranging (table 1). The adult beetles were bright yellow in colour immediately after emergence and gradually turned to greyish brown. It was also observed that adult beetles were sluggish in nature and fly occasionally. The adult females were slightly larger than the male counterpart. Females had a longer lifespan (21.10 days) than the males (16.38 days). In present study, five overlapping generations were noted as against nine overlapping generations in a year on waternut crop as reported by Srivastava [20] from Kanpur, Uttar Pradesh and this could be the differences in ecological condition of the region. The study was mainly aimed at recording of different biological parameters of *G. birmanica* in its habitat Varanasi, Uttar Pradesh. There is a possibility of change in the growth and developmental parameters when an insect occupies a new area [21].

It is evident that under Varanasi condition fecundity per gravid female and incubation period of *G. birmanica* were slightly lower (90.4 ± 14.67 per gravid female) to those reported by Srivastava *et al*. [22] from Kanpur, Uttar Pradesh (52-203 eggs per gravid female). Similarly, there is also slight variation in larval, pupal and adult durations amongst the populations of Navsari, Gujarat and Kanpur, Uttar Pradesh, India. The variation in different biological parameters compared to different researchers might be due to the different geographical condition [23].

Table 1: Biological events in life-cycle of *G. birmanica* on water chestnut under laboratory conditions

Biological parameters	Minimum	Maximum	Mean* ± SD
Fecundity (Nos.)	73	111	90.4 ± 14.67
Egg viability (%)	53	64	58.9 ± 3.75
Incubation period (days)	3.50	5.50	4.15 ± 0.56
Larval duration (days)			
First instar	3.25	4.25	3.68 ± 0.39
Second instar	4.50	5.25	4.85 ± 0.36
Third instar	4.00	4.75	4.28 ± 0.38
Total larval period	11.75	14.00	12.75 ± 0.80
Pre-pupal period (days)	2.50	3.25	2.80 ± 0.40
Pupal period (days)	3.50	4.75	3.98 ± 0.52
Adult longevity (days)			
Male	15.50	17.25	16.38 ± 0.77
Female	19.00	23.75	21.10 ± 1.57

SD= Standard Deviation; *Means are based on thirty replications

3.3 Bioassays

Amongst the three entomopathogenic tested, green muscardine fungus *M. anisopliae* IIVR strain was found most promising against the second instar grubs of *G. birmanica* followed by *B. bassiana*. IIVR strain of *M. anisopliae* took 52.67 h to kill the 50 per cent test population whereas *B. bassiana* had 58.88 h. Moreover, Neem oil (1%) had showed expressive superiority among all the biopesticides tested in terms of manifesting lowest median lethal time (LT_{50}) of 17.32 h. however, all these microbials insecticides when blended with neem oil (1:1 ratio) at their half-doses were found compatible and synergistic in nature. Combination of *M. anisopliae* and Neem oil had registered lowest median lethal time of 11.15 h followed by *Beauveria bassiana* + Neem oil (LT_{50} = 12.91 h). Pathogenicity of *M. anisopliae* against coleopteran insects had been confirmed by several authors in the past. *M. anisopliae* strains were pathogenic to all development stages of red palm weevil, *Rhynchophorus ferrugineus* causing up to 80–100% mortality of larvae and adult weevils under laboratory conditions [24]. Native strain of *M. anisopliae* was found most potent against the adults of *Epilachna duodecastigma* (Wied.) infesting organically grown cowpea by recording lowest LT_{50} value of 60.86 h [25]. Similarly, compatibility between these entomopathogenic fungi and botanicals like neem oil was also confirmed by several authors [26, 27, 28]. Many botanical insecticides including Azadirachtin is having diverse mode of action. The apparent enhancement in activity of neem oil and entomopathogenic fungi mixtures were attributed to the possible additive, synergistic and/or stabilizing effect of neem oil [29, 30].

Table 2: Median lethal time of entomopathogenic fungi and neem oil alone and their 1:1 combinations against adults of *G. birmanica*

Treatments	Heterogeneity		Regression equation (Y=)	Median lethal time (LT_{50}) (h)	Fiducial limit
	df	$\chi 2$			
Beauveria bassiana	4	0.630	6.592X – 0.667	58.88	67.65 – 51.25
Metarhizium anisopliae	5	1.467	2.299X + 1.043	52.67	71.02 – 39.06
Lecanicillium lecanii	5	0.398	4.128X – 0.270	73.33	88.81 – 60.55
Neem oil (1%)	4	5.723	3.867X + 0.210	17.32	30.08 – 9.98
Beauveria bassiana + Neem oil (1:1)	5	0.691	1.581X + 0.332	12.91	66.20 – 25.18
Metarhizium anisopliae + Neem oil (1:1)	5	1.174	1.590X + 0.334	11.15	68.48 – 18.16
Lecanicillium lecanii + Neem oil (1:1)	4	1.926	3.032X + 1.338	16.15	21.35 – 12.21

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