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Distribution, Host Range and Bionomics of *Rhopalosiphum nymphaeae* (Linnaeus, 1761), a Polyphagous Aphid in Aquatic Vegetables

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ABSTRACT

The present study records the occurrence of water lily aphid, Rhopalosiphum nymphaeae (Linn, 1971) on different aquatic plants from Varanasi and adjoining regions, Uttar Pradesh, India for the first time. Both nymphs and adults prefer to feed on petiole, leaf lamina and buds towards terminal portion of different aquatic plants. They suck the cell sap resulting in curling of leaves, stunted plant growth with lower number of fruits in water chestnut. Further, the honey dew deposited on the leaves favours the development of black sooty-mould and hinders the normal photosynthetic activity of the plants. Infestation of aphid started in the region on water chestnut during last week of October (1.35 aphids leaf¹) coinciding with the initiation of winter in the region and gradually increased with highest peak (21.95 aphids leaf¹) during fourth week of January. Amongst the different aquatic host plants under study in the region, olfactometer study showed significantly highest number of R. nymphaeae (28%) attraction towards lotus followed by of water chestnut (21.3%) and water lily (20%) and the least was with water hyacinth (4%). Amongst the biopesticides tested, L. lecanii @ 5 g lit¹was found most promising with lowest median lethal time (31.09 h) followed by neem oil (1%) (41.59 h) and *M. anisopliae* (43.95 h).

Keywords: Rhopalosiphum nymphaeae; Host preference; Seasonal incidence; Management

INTRODUCTION 1.

Water lily aphid, Rhopalosiphum nymphaeae (Linnaeus, 1761) (Hemiptera: Aphididae), is a polyphagous heteroecious holocyclic aphid and reported to feed on a large variety of host plants including many aquatic plants. This aphid is also recorded to survive underwater conditions¹. This polyphagous species is known to be a polymorphic as it produces different morphs in temperate conditions whereas parthenogenetic apterous and alate viviparous females in plains of India². In large aphid colonies that develop on water lilies, aggregate along the leaf veins and infest flowers as well. The sexual forms have been reported on Prunus spp.³. In Makhana (Euryale ferox Salisb.) the yield loss was to the tune of 17.04 to 23.66 per cent due to its insect pests including R. nymphaeae in Purnea, Bihar, India during the summer seasons of 2015-16⁴. The rates of development, natality and survivorship of R. nymphaeae have been studied in relation to its potential for virus transmission on both crops and aquatic weeds⁵.

Recently, large colonies of this aphid were observed to infest many aquatic vegetables including water chestnut, water spinach, lotus, water lily, etc. in and around Varanasi region of Uttar Pradesh, India. In these water vegetables of the region, they feed on leaf petioles, leaf lamina and fruit stalks resulting in curling of the leaves, stunted growth of the plants

and lower number of fruit settings. Apart from abstracting cell sap, they also secret the copious amount of sugar rich honey dew which is deposited on the plant surface favouring the development of black sooty-mould and there by hindering the normal photosynthesis of the plants^{6,7}. Several local farmers from Varanasi and adjoining Mirzapur district also confirmed severity of the problem of aphids in the region. This prompted to conduct a detailed study on this nefarious pest pertaining to its distribution, bionomics, damage and potential biocontrol agents for the pest management in agro-climatic condition of Varanasi region of eastern Uttar Pradesh.

2. MATERIALS AND METHODS

Detailed studies on morphology, nature of damages, feeding preference along with search for suitable bioagents and plant origin insecticide for its management were carried out in the Entomology laboratory of ICAR- Indian Institute of Vegetable Research, Varanasi (82°52' E longitude and 25°12' N latitude), Uttar Pradesh, India whereas the studies on seasonal incidence was carried out during the April to March of 2017-18 at the nearby villages viz., Kelabela and Shahanshahpur, Uttar Pradesh, India.

2.1 Taxonomic Identification

The aphid specimens were collected and preserved in 90 per cent alcohol were used for taxonomic identification.

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2.2 Studies on Nature of Damage

To study the nature of damage, fresh uninfested water chestnut plants were brought form the nearby ponds/water bodies of the villages Shahanshahpur and Kelabela, Uttar Pradesh and placed in water filled plastic jar (11 cm dia and 13.6 cm height) in the biocontrol laboratory under $27\pm1^{\circ}$ C temperature and $70\pm5\%$ relative humidity (RH). Fifty first instar *R. nymphaeae* were released on each plant and observations on nature of damage on these plants were made at regular intervals. Apart from these, observation was also taken from the water chestnut grown in ponds infested with the aphids at periodical intervals.

2.3 Studies on Seasonal Incidence

To study the seasonal incidence of *R. nymphaeae* on water chestnut, number of aphids (both nymphs and adults) on each leaf was counted at weekly intervals initiating with its first occurrence till end of the season when the population vanished on water chestnut. Total sixty leaves from three ponds/water bodies were randomly taken for each observation and its population expressed as number of aphids leaf¹.

2.4 Studies on Feeding Preference

Similarly, to ascertain the feeding preference of the aphid, aquatic plants commonly grown in the region viz., water lily (Nymphaea rubra Roxb. ex Salisb.), water chestnut (Trapa natans Linn.), water spinach (Ipomoea aquatic Forssk.), lotus (Nelumbo nucifera Gaertn.), water hyacinth (Eichhornia crassipes (Mart.) Solms) and azolla (Azolla pinnata R.Br.) were selected. Two tender leaves of each plant (in case of water chestnut and water spinach) were used in the experiment. In case of lotus, water lily and water hyacinth, leaf size of 4 x 4 cm was cut into pieces and two such samples were kept in the simple olfactometer where as in case of Azolla, being small in size, five such plants were selected for the experiment. All these plant and plant parts as well as the sap suckers were subjected to free-choice arena (olfactometer) test. All plants mentioned as above were subjected to a six armed olfactomenter assays with aphids. The olfactometer had a central "arena" (18.7 cm diameter \times 5.1 cm depth) and six equidistantly placed arms (2.5 cm diameter and 9.5 cm length). Fifty second instar nymphs of R. nymphaeae, starved for 6 h, were released in the middle of the arena. After 3 h, the numbers of aphids alighting on each particular plant were counted. Three replicates for each study were maintained and the preference of the aphids was expressed as per cent.

2.5 Bioefficacy of Entomopathogens and Botanical Insecticide

For its ecofriendly management of this aphids in aquatic environment, talc based formulations of three entomopathogenic fungi (EPF) viz., Beauveria bassiana IIVR strain $(1 \times 10^{10}$ cfu/g), Metarhizium anisopliae IIVR strain $(1 \times 10^{10}$ cfu/g) and Lecanicillium (=Verticillium) lecanii (2×10^8 cfu/g) were used for the experiments. Neem oil (1%) was prepared by dissolving in emulsifying water containing Triton X-100 as an emulsifier. All the EPF (5 g/l of water) including untreated control were sprayed alone under Potter's tower at 340 g/cm² pressure. In addition, these entomopathogens and neem oil were also mixed at 1:1 ratio and 1 ml of such mixtures was sprayed under Potter's tower. Fresh uninfested and untreated tender water chestnut leaves were given to the treated insects as food and kept at biocontrol laboratory under 27±1°C temperature, 70±5% relative humidity and a photoperiod of 13:11 (L:D) hour. Twenty second instar nymphs comprised of a replication and as such three replications were maintained for each treatment. For the assessment of toxic effect, mortality counts were taken at every 12 h interval and moribund insects were considered as dead. The mortality data was corrected by Abbott's formula⁸ and analyzed by Probit analysis⁹ with SAS program (version 9.2). The control mortality, in almost all the cases, was below 10%. The median lethal times (LT_{50}) were determined and any two values were considered significantly different if their respective 90% confidence limits (CL) did not overlap.

3. **RESULTS AND DISCUSSION**

3.1 Taxonomic Characters of *Rhopalosiphum nymphaeae*

This species comprises both apterous and alate viviparous females in the area.

Apterous viviparous females: Reddish brown to dark olive, dusted with grey wax. Body 1.3-2.6 mm long. Head dark brown, with low lateral frontal tubercles. Antennae 6-segmented, about 0.57-0.75X body, processus terminalis 3.0-5.7X the base of segment VI. Ultimate rostral segment 1.0-1.2X the hind tarsal segment. Abdominal tergites with spinules arranged in polygons and each polygon encloses a central group of spinules; longest seta on anterior tergites 0.7-1.0X the basal diameter of antennal segment III; tergite VIII with 2 setae; marginal tubercles distinct and always present of segment I and VII. Siphunculi dark brown, slightly swollen, about 0.13-0.18X the body length and 1.65- 1.83- 2.14X the cauda which is with a basal constriction and bears 4-8 hairs.

Alate viviparous females: shiny black and dark brown with a whitish bloom on ventral surface of body. Body 1.74-1.87 mm long. Head dark brown. Antennae 0.74-0.81X the body length; processus terminalis about 3.0-3.9X the base of segment VI; segment III with 14-18, IV with 3 accessory sensoria. Abdominal dorsum pale, with pale brown marginal sclerites. Siphunculi pale brown, swollen on apical half, about 0.17X the body length and 2.25X the dusky cauda¹⁰.

3.2 Nature of Damage

Large aphid colonies comprising both nymphs and adults were found to congregate on petiole, leaf lamina and buds of water chestnut towards terminal portion of the plant. Both nymphs and adults suck the cell sap resulting in the curling of leaves. In severe infestation, growth of the plants was stunted with few small size curled leaves. Affected plants also harbored less number of fruits. In case of water lily and lotus, aphid colonies were noticed on leaf lamina along the leaf veins and infest flowers, as well. Due to their infestation, the leaves become small in sized and curled upward. Honey dew deposited on the leaf surfaces favoured the development of black sooty-mould which further hinders the normal photosynthetic activity of the plants. Both parthenogenetic apterous and alate viviparae form were prevalent the region. Unlike some other aphids, no symbiotic association with ants was observed with R. nymphaeae throughout the period of observation. Aquatic and semi-aquatic nature of the host plants could be the probable reason for the absence of ants in attending R. nymphaeae. Water lily aphid can readily walk on the water surface in search of suitable hosts and use their stylet to probe plants before selecting a host¹¹. After colonising aquatic sites, aphids reproduce rapidly, often virtually blanketing the hydrophytes present. The water lily aphid is extremely destructive and can transmit at least five plant viruses^{5,12}.

3.3 Seasonal Incidence

From Fig. 1, it is evident that aphid population initiated on water chestnut from last week of October (1.35 aphids leaf¹) coinciding with the initiation of winter in the region. Its population gradually increased from November to till January with a highest peak (21.95 aphids leaf¹) during fourth week of January (Fig. 1). An untimely rain lashed out during last week of January and the maximum population of R. nymphaeae washed away leading to the drastic reduction of aphid population during last week of January (9.55 aphids leaf⁻¹). From March onwards summer started in the region and population of aphids on water chestnut gradually declined. From second week of April onwards its population was nil on water chestnut and other aquatic plants prevailing in the region. R. nymphaeae being heteroecious, move between a woody primary host and an herbaceous secondary host¹². Over-crowding, competition and feeding resources may all increase aphid mobility^{11,12}. In the present study, aphids did develop alate individuals when the colonies became overcrowded. Biswas and Ghosh (1981) reported this species as widely distributed worldwide and is known to overwinter on Rosaceous host like Plum and other Prunus spp. and spend the summer in aquatic or semi aquatic hosts in Nearctic and Palearctic regions.

3.4 Host Preference

Among the different host plants analysed for host preference with the help of a six-armed olfactometer, significantly the highest number of *R. nymphaeae* (28%) were attracted towards the young leaves of lotus followed by leaves of water chestnut (21.3%) and water lily (20%). So, their

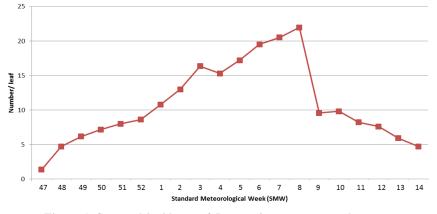
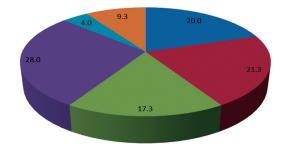


Figure 1. Seasonal incidence of R. nymphaeae on water chestnut.

descending order of attraction/preference towards different hosts was lotus > water chestnut > water lily > water spinach > azolla > water hyacynth (Fig. 2).

3.5 Bioefficacy of Entomopathogens, Botanical Insecticide

Amongst different biopesticides the tested. entomopathogenic fungus viz., L. lecanii was found most promising against the second instar nymphs of R. nymphae under laboratory conditions. Maximum 61.09 and 74.25% mortalities were recorded at 48 and 72 h after the treatment (HAT) followed by M. anisopliae and the corresponding values were 55.65 and 64.46%, respectively (Fig. 3). The neem oil (1%) had also shown lethal activity against this polyphagous pest and per cent mortalities were 53.28 and 66.27% after 48 and 72 HAT. Blending (1:1 ratio) of these EPF with neem oil at half of their recommended doses were found compatible and synergistic activity as evident in their mortality. Combination of L. lecanii and neem oil at half of



■ Water Lily ■ Water Chestnut ■ Water Spinach ■ Lotus ■ Water hyacynth ■ Azolla

Figure 2. Free-choice arena test with six host plants and *R. nymphaeae*.

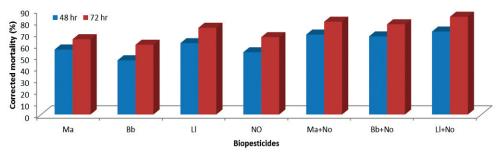


Figure 3. Bio-efficacy of different biopesticides against R. nymphaeae.

Biopesticides	Heterogenity		Regression equation	LT (hr)	Fiducial limit
	df	χ^2	(Y=)	LT ₅₀ (hr)	Fluuciai iiiiit
Metarhizium anisopliae	6	0.524	2.570X - 0.777	43.95	53.69 - 35.98
Beauveria bassiana	7	0.512	2.792X - 0.212	51.90	64.81 - 41.56
Lecanicillium lecanii	5	2.408	2.210X - 1.701	31.09	39.27 - 24.62
Neem oil (1%)	6	2.499	2.519X - 0.922	41.59	50.71 - 34.10
Metarhizium anisopliae + Neem oil	5	0.402	2.438X - 1.509	27.03	34.45 - 21.22
Beauveria bassiana + Neem oil	5	0.676	1.990X - 0.932	36.04	45.81 - 28.36
Lecanicillium lecanii + Neem oil	5	0.903	3.189X - 0.622	23.60	29.40 - 18.94

Table 1. Bio-efficacy of different EPF alone and in combination with neem oil (1:1) against R. nymphaeae

their recommended doses was registered highest nymphal mortality both 48 (71.04%) and 72 HAT (83.69%).

Amongst the different biopesticides tested, L. lecanii was found most promising and registered lowest median lethal time (31.09 h) followed by neem oil (41.59 h) and M. anisopliae (43.95 h) (Table 1). When, these three EPF were mixed with neem oil at 1:1 ratio and sprayed at half of their recommended doses, L. lecanii and neem oil showed their highest compatibility and synergistic activity against the adults of *R. nymphaeae* as evidenced by their lowest median lethal time of 23.60 h amongst all the treatments (Table 1). However, other two EPF i.e., M. anisopliae and B. bassiana were also promising against this water lily aphid. From our present investigation, it is evident that white halo fungus, L. lecanii apart from controlling different sucking insect pests, also controls this aquatic aphid. Moreover, they are also compatible with plant origin insecticides like neem oil. In another study, it was found that entomopathogens, B. bassiana and V. lecanii were promising against major sucking pests of vegetables viz., Phenacoccus solenopsis, Aphis craccivora and Lipaphis erysimi¹³. Compatibility of neem with major entomopathogenic fungi was ascertained by several researchers in the past^{14,15}. The present study also confirmed the compatibility of neem oil and entomopathogenic fungi at 1:1 ratio and these could be a better biorational option for integration in IPM programme.

4. CONCLUSIONS

Water lily aphid, Rhopalosiphum nymphaeae was recorded as a serious sucking pest of different aquatic plants from eastern Uttar Pradesh, India. Infestation of aphid started in the region on water chestnut during last week of October coinciding with the initiation of winter in the region and gradually increased with highest peak during January. Amongst the different aquatic host plants under study in the region, highest (28%) host preference was towards lotus followed by of water chestnut (21.3%) and Water lily (20%). Amongst the biopesticides tested, L. lecanii at recommended dose was found most promising with lowest median lethal time (31.09 h) followed by neem oil (1%) (41.59 h) and M. anisopliae (43.95 h). Compatibility and synergistic activity of these entomopathogenic fungi and neem oil at half of their recommended doses were also established against this polyphagous aphid.

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CONTRIBUTORS

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In the present study, he carried out the experiments on seasonal incidence, insect toxicology, studied the host preference oh *R. nymphaeae* under olfactometer, collection of literature and preparation of the manuscript.

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His contribution includes supervision of the work, statistical analysis of the experiments and correction of the manuscript.

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In the present study, she helped in identifying the insect specimens, collected the related literature, designing the experiments as well as preparation of the manuscript.