# PARTIAL PURIFICATION AND ELECTRON MICROSCOPY OF PERGULARIA MOSAIC VIRUS

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*PERGULARIA MINOR* Andr. (Asclepiadaceae) is an ornamental climber. A mosaic disease on *Pergularia* was first reported by Eranna and Nayudu[1]. The causal virus induces systemic symptoms in *Pergularia minor*, *Cyamopsis tetragonoloba* and *Crotolaria juncea*, and local lesions in *Chenopodium amaranticolor*. The present note reports the partial purification and electron microscopy of the virus.

Stock culture of the virus was maintained on *Pergularia* plants by rooting the stem cuttings from infected plants in 30 cm pots kept inside the insect proof 40 wiremesh house. *C. amaranticolor* was used for assaying the virus.

Infected *Pergularia* leaf material was harvested, weighed and washed with 0.01 M, phosphate buffer pH 8, and ground in cold using the above buffer containing 0.05% 2-mercaptoethanol. The extract was squeezed through two layers of muslin cloth. Carbon tetrachloride (10% by volume) was added to the filtrate, shaken well, and kept in a refrigerator for 15 min with intermittent shaking, and centrifuged at 8000 rpm for 15 min and the aqueous layer separated. The virus in the aqueous phase was precipitated by the addition of polyethylene glycol 6000 (4% *PEG*) and sodium chloride (0.2 M) and then centrifuged. The pellets, obtained after centrifugation (8000 rpm, 10 min) were resuspended in phosphate buffer (without 2-mercaptoethanol) and clarified by centrifugation. The supernatant was acidified with 0.1 N acetic acid to pH 5 and kept in a refrigerator for 10 min and centrifuged as above. The sediment was washed with 2-methoxyethanol, resuspended in phosphate buffer and centrifuged. The supernatant was used for taking UV absorption spectrum and for electron microscopy.

The UV absorption spectrum of the virus was taken in Beckman $DU_2$ spectrophotometer. Purified virus stained in neutral 2% sodium phosphotungstate was observed in Phillips 201C model electron microscope.

Herbert[2] reported 4% PEG + 0.3 M NaCl for the precipitation of rod shaped viruses and 8% PEG + 0.3 M NaCl for spherical viruses. The present virus precipitated with 4% PEG + 0.2 M NaCl, gave highest infectivity when compared to other concentrations of

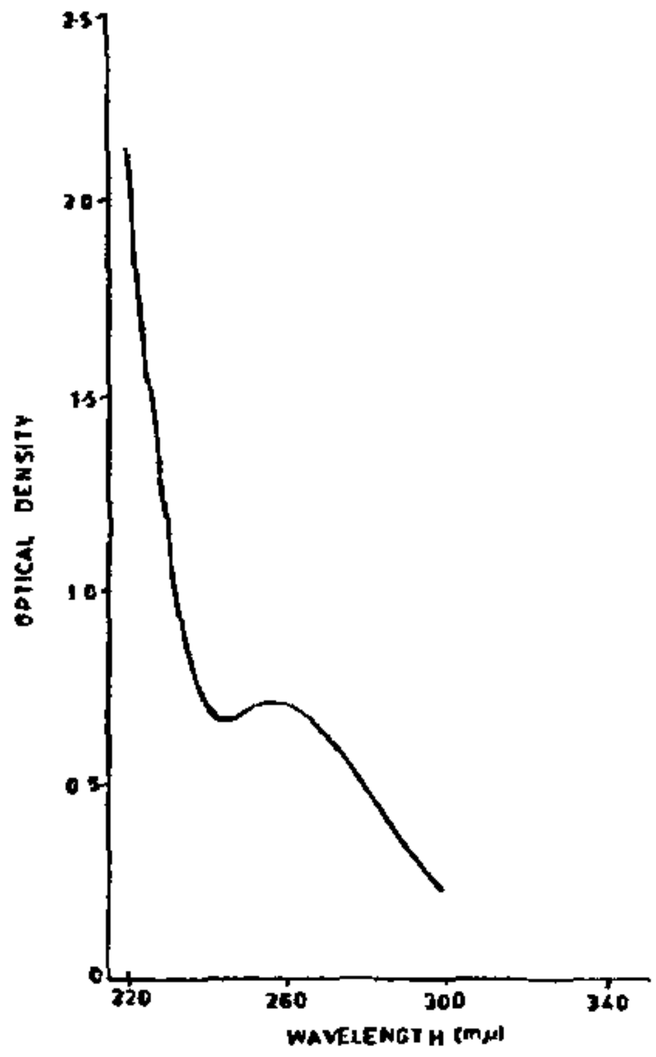


**Figure 1.** Ultraviolet absorption spectrum of purified virus.

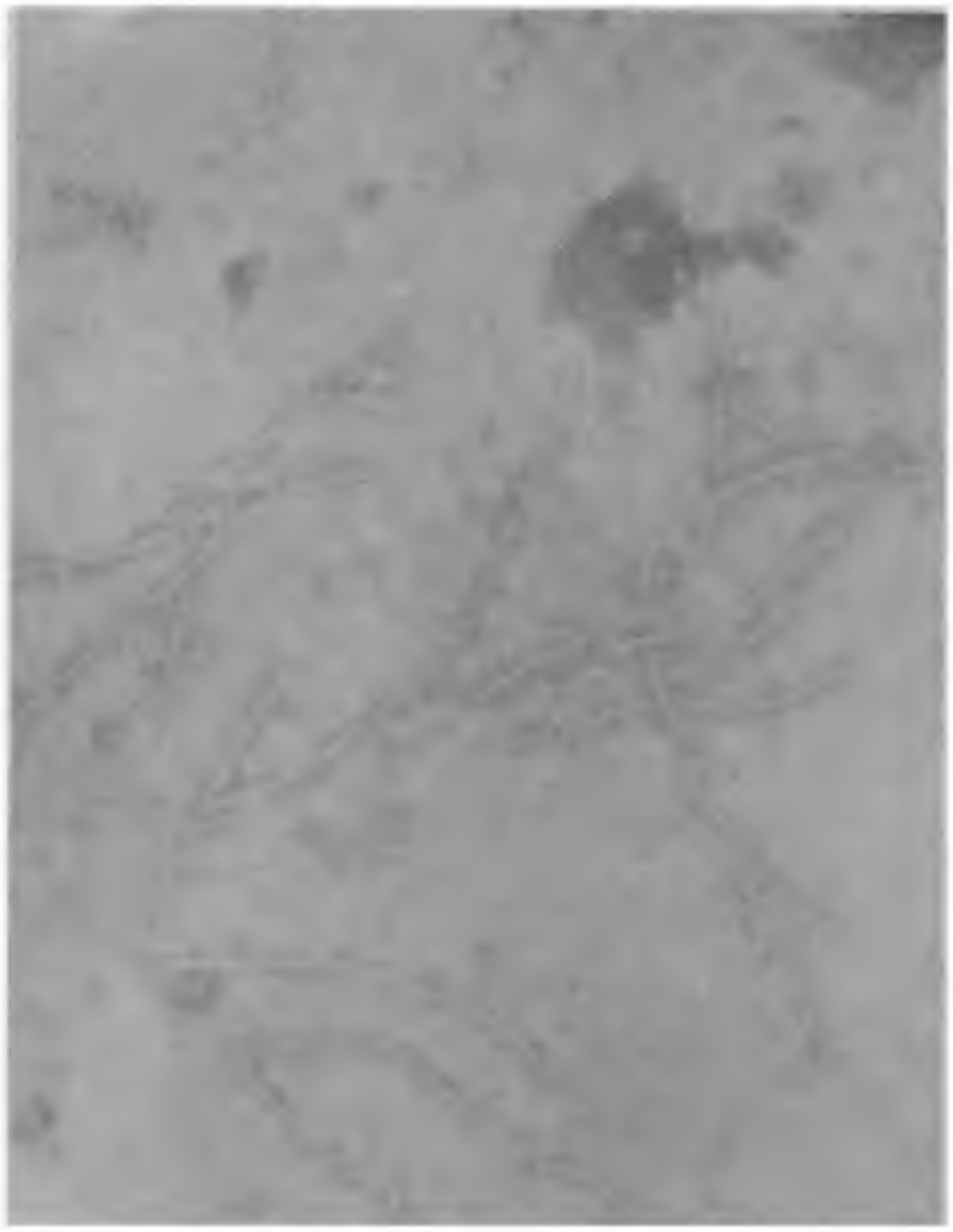

**Figure 2.** Negatively stained flexuous filamentous virus particles purified from *Pergularia* leaves.

PEG. The principle of minimum virus solubility at and near its isoelectric point is used in virus precipitation by acidification. The virus precipitated with acetic acid gave almost typical UV absorption spectrum (figure 1) as compared to potyviruses. The ratio of absorption at 260 nm and 280 nm of 1.29 indicated that the virus contained about 5% nucleic acids[3]. Flexuous filamentous particles measuring c.730 nm long (figure 2) were observed, a characteristic feature for the members of potyvirus group[4].

Pergularia mosaic virus resembles members of potyvirus group in its shape and length, thermal inactivation point (58°C for 10 min), dilution end point (1:200), and longevity *in vitro* (30 hr at 30–35°C and 23 days at 10–15°C). It resembles a potyvirus of milkweed vine virus in particle morphology and physical properties but the host range of milk weed vine virus is limited to 6 genera in the Asclepiadaceae[5], whereas the present virus has the host range outside the Asclepiadaceae[1]. The present virus was precipitated without losing its infectivity by 4% PEG + 0.2 M NaCl as of many potyviruses. Hence the present virus is a member of potyvirus group.

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## *SPODOPTERA LITURA* AS A BIOCONTROL AGENT OF *EICHHORNIA CRASSIPES*

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WATER hyacinth infestation is an alarming problem to water reservoirs. Kerosene burners to laserbeams[1] have been conceived as a means of control to eradicate this weed. Chemical control of this weed by means of herbicides, weedicides and fungicides has proved to be expensive and toxic to the environment, hence natural control of the weed has gained importance.

Biological control of water hyacinth necessitated a search for natural enemies because of its long-term benefits[2]. A biocontrol agent once established, becomes an integral part of the environment and as such is considered to be a self-renewable source[3]. Bennett[3] has listed many of the polyphagus pests as natural enemies to water hyacinth. In this paper authors report the observations on another polyphagous pest, *Spodoptera litura* which has been found to infest water hyacinth in the natural environment.

Field surveys showed that stands of *Eichhornia crassipes* (water hyacinth) were heavily infested by the larvae of *S. litura*. The damaged plants from the fields were collected and brought to the laboratory to assess the extent of damage and to study the life cycle of the insects. Parallel experiments with laboratory maintained cultures of *S. litura* were also run by introducing these insects to small areas of water hyacinth plants. The adult moths were released into cages of about 4.5 ft in length, 3 ft width and 4 ft height containing hyacinth plants. The cages were covered with polythene net on all sides leaving a provision to catch the adults and larvae. The adults were observed to oviposit on fresh foliar leaves. The eggs hatched into 1st instar larvae within three days. The newly hatched larvae caused superficial damage to the leaves, but as they grew in size they fed voraciously and tunnelled into the petioles and entered into the prepupal stage after 12–14 days. At this stage, feeding was totally stopped and the dormant larvae pupated in the tunnels and underwent an incubation period of five days before they emerged into adults. Adults were collected for further rearing and the damage caused by the larvae were assessed and the results are presented in the photographs.

Since field observations revealed extensive damage by *S. litura* on water hyacinth, experiments were designed to study its life history and its role as a biocontrol agent. Some investigators[1,2,4] have shown that the lepidopteron pests like *Sameodes albiguttalis*, *Arzama densa* and *Acigona infusella* also caused considerable damage on water hyacinth. Our studies revealed that the larval stages of this lepidopteron moth caused 20–25% damage (figure 1).

The sites of these larval attacks were good sources for secondary fungal infections which were studied for their pathogenicity[5]. It is known that pathogenic and