

## Record of *Ptyomaxia syntaractis* Turner, 1904 (Lepidoptera: Pyralidae) as a Major Insect Pest of *Avicennia marina* and Detection of Bacterial Pathogen *Myroides odoratus* in the Mangroves of Maharashtra

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### ABSTRACT

*Ptyomaxia syntaractis* Turner, causes regular defoliation on the mangroves of Mumbai region particularly in Airoli creek of Navi Mumbai areas of Maharashtra. This pest is prevalent during the post monsoon period and infesting *Avicennia marina* severely in Airoli mangroves. The assessment of intensity of infestation revealed 64 to 70% infestation from September to November. The biology of the pest is studied in the laboratory. Also natural occurrence of the bacterial pathogen *Myroides odoratus* is observed for the first time on *P. syntaractis* in field condition. The pathogen was identified carrying out the method Genomic DNA isolation, PCR amplification using universal 16s rDNA primers and DNA sequencing. Pathogenicity test confirmed the infectivity of the bacteria on *P. syntaractis* in laboratory condition.

**Key words:** *Ptyomaxia syntaractis*, Mangroves, Pathogen, Intensity, Assessment

### INTRODUCTION

*Avicennia marina* (Forssk.) Vierh. is the most common and dominant species of mangroves available in all the mangrove areas in India (Kathiresan & Rajendran, 2005), which was found infested by the defoliating insect pests during the rainy season particularly during the post monsoon period (Raja Rishi & Sundararaj, 2020). Raji (2003) reported 340 species of insects belonging to 11 orders in the west coast of South India. Swetha et al. (2019)

updated the list of entomofauna of mangroves in India which indicated about 516 insect species belongs to 111 families. *Ptyomaxia syntaractis* Turner, 1904 (Lepidoptera: Pyralidae) is a polyphagous pest infesting on mangrove species including *A. marina*, *A. officinalis* and *Rhizophora* sp. Anderson and Lee (1995) reported this pest on *A. marina* in the mangroves at the Mai Po Marshes, Hong Kong.

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*P. syntaractis* caterpillars graze leaves on the underside leaving the upper epidermis and veins intact (Allen, 1984), a mode of feeding which enables the degree of leaf damage to be estimated reliably. Young larvae also burrow into bud tissues to feed and eject faecal matter from the tunnels formed. Considering the importance and severity of this pest on mangroves a study was conducted to record the bionomics of this defoliating pest in mangrove ecosystem of Maharashtra. Surveys conducted to assess the pest problems of mangrove species in Airoli and Vashi creek revealed the severe infestation of *P. syntaractis* on *A. marina* during the year 2018. The study also resulted in detection of a species of native pathogenic bacteria, *Myroides odoratus* comb.nov. in *P. syntaractis*. Therefore, an attempt was made to

assess the potential of this native pathogenic bacteria for the control of the this defoliator in laboratory condition.

## MATERIALS AND METHODS

Periodical surveys were conducted in mangrove areas of Airoli ( N 19° 14' 76.5" E 072° 98' 43.9"), Ghansoli mangrove plantations ( N 19° 11' 50.9" E 072° 99' 17.3"), and Gorai ( N 19° 24' 04.8" E 072° 80' 08.8") (Fig 1), from 2018 to 2019 to record the pest status of *P. syntaractis*. The intensity of infestation was assessed based on the level of incidence of the insect pest and percentage of the damage/extent of damage caused. Larvae collected from the field were reared in laboratory condition and its life cycle was studied.

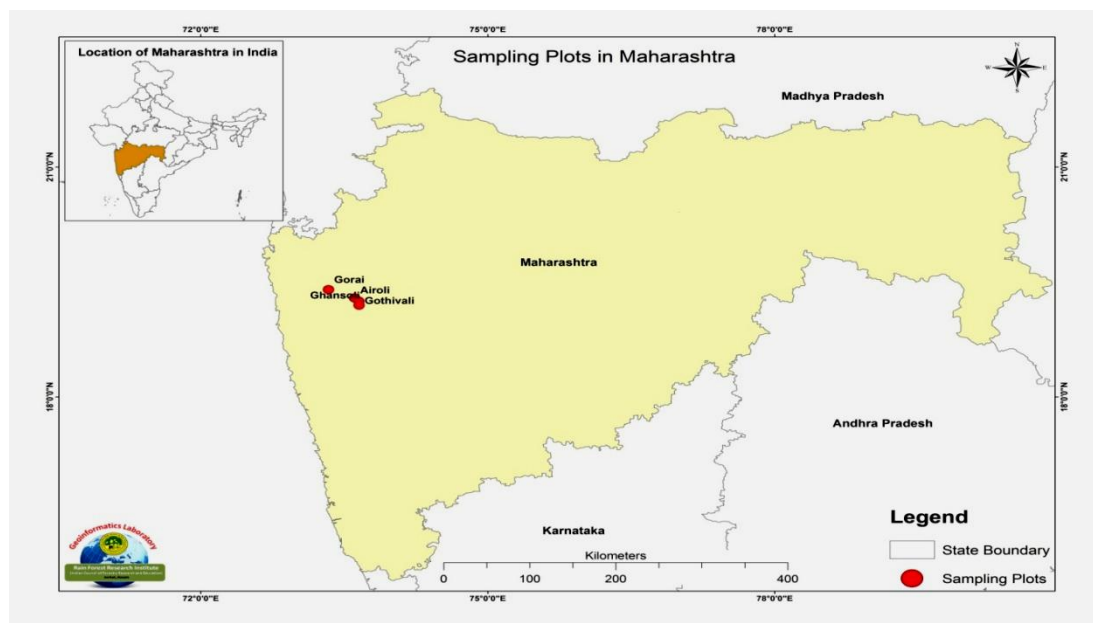


Fig. 1: Study areas

Also since we observed few dead larvae of *P. syntaractis* in field, the cadavers were brought to the laboratory and screened for the infection.

### Isolation of bacterial strain:

The field collected cadavers of *P. syntaractis* were sterilized by immersing in 95% ethanol for 30-60s and flaming off the alcohol (Jackson et al., 1995). The gut contents of the larva were aseptically excised and the

suspension was poured on nutrient agar. The plates were incubated aerobically at 32°C for 24 hours. The bacteria with characteristic yellow pigmentation were Gram stained, sub cultured on nutrient agar plates and stored in 80% glycerol solution, following the standard protocol (Dharne et al., 2008). The identification of the isolated bacterial strain was carried out by molecular characterisation and phylogenetic analysis by 16s rRNA gene

sequence of the bacterial strain using PCR and DNA sequencing protocols by Juniper Life Sciences, Bengaluru.

#### Pathogenicity test:

The test insect *P. syntaractis* were subjected to pathogenicity test to confirm the infectivity of the bacteria in laboratory condition. The bacterial strain were grown on nutrient agar medium at the temperature 32°C for 3 days and were harvested, and stock solution was prepared with distilled water. The stock solution of the pathogen was serially diluted from  $10^{-1}$  to  $10^{-9}$  and effective colony forming dilutions  $2.6 \times 10^8$  CFU/ml,  $2.6 \times 10^6$  CFU/ml and  $2.6 \times 10^4$  CFU/ml were determined to test the efficacy against *P. syntaractis*. A few drops of teepol/soap solution was added as wetting agent to the effective dilutions of the strain and mixture of the same was sprayed

over *A.marina* leaves with the help of fine sprayer and air dried in shade. Third instar larvae selected from the stock culture maintained in the laboratory were fed separately for first two days and thereafter fresh contaminated leaves were provided every day for feeding. Five replicates with 10 larvae each were used for each experiment. Another set of 10 larvae were fed with fresh leaves treated with teepol mixed with sterile distilled water which served as the control. Observations on larval mortality, weight loss/gain and feeding behavior of the larvae were recorded at 24hrs intervals. Observations on the larval mortality of insects were recorded at different time duration at different concentrations and percent larval mortality was calculated using Abbott's formula (Abbott, 1925).

$$\text{Percentage larval mortality} = \frac{\% \text{ treated mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$

## RESULTS AND DISCUSSION

The defoliator *P. syntaractis* is observed as a seasonal and major pest in Airoli and Ghansoli

mangrove areas. Its intensity of infestation on *A. marina* ranged from 64 to 70% during the months from September to November (Fig 2).

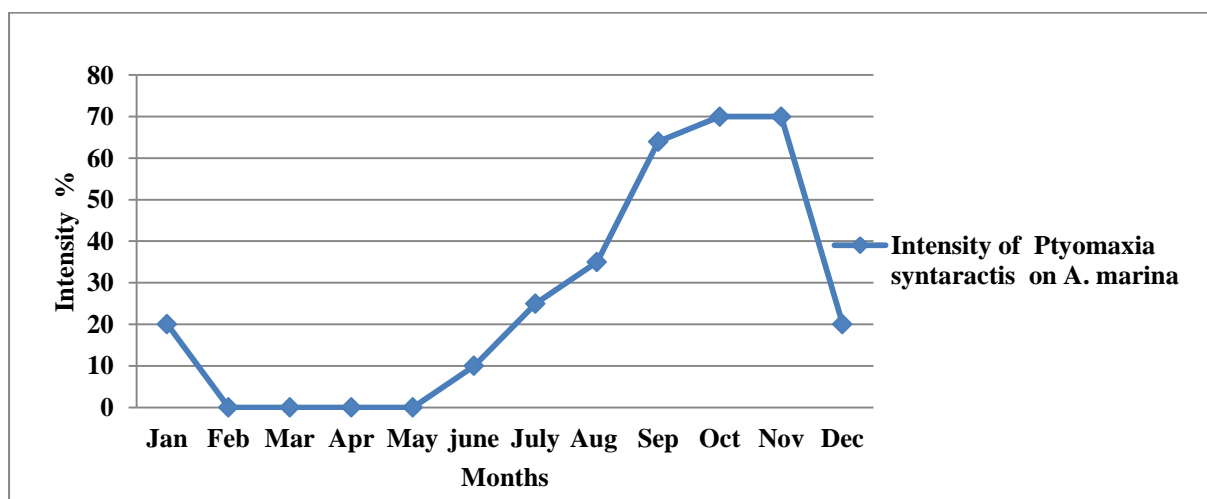


Fig. 2: Intensity of *Ptyomaxia syntaractis* on *A. marina*

The larva feed on the young shoots and skeletonize the leaf of *A. marina* and *A. officinalis* and cause defoliation in nurseries, young plantations and in natural mangroves. The full grown larva is bluish green in colour (Fig 4 A). Larval period is 13 to 15 days. Pupa occurs in a cocoon between the

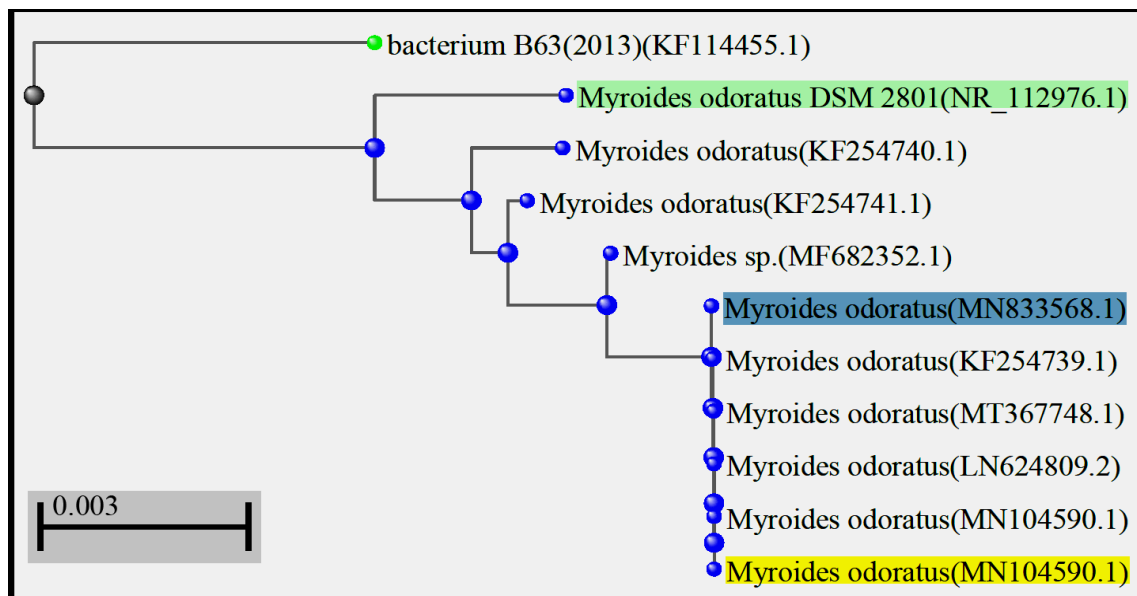
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leaves. The greyish, pale white adult moths are having 22 to 28mm wing span (Fig 4 B & C). Life cycle completes within one month period.

The bacterial strain isolated from the infected cadaver of *P. syntaractis* was identified as *M. odoratus* by carrying out the

method Genomic DNA isolation, PCR amplification using universal 16s rDNA primers and DNA Sequencing the PCR product using ABI Sequencing machine. The 16s rRNA gene sequence of bacterial strain was deposited to genebank and was compared with the sequences obtained from GenBank. Macroscopic examination on solid media showed well-isolated colonies. 16s rRNA gene sequence of the bacterial strain (GenBank

accession number MN104590.1) showed highest similarities to the sequences of *M. odoratus* (MN104590.1) (100%), *M. odoratus* (LN624809.2) (100%), *M. odoratus* (MT367748.1) (100%), *M. odoratus* (KF254739.1)(100%) and *M. odoratus* (MN833568.1) (100%). The phylogenetic tree showed that the strain clustered with *M. odoratus* with 100% bootstrap support (Fig 3).



**Fig. 3: Phylogenetic affiliation of *Myroides* sp. strain isolated from *P. syntaractis***

The efficacy of native pathogenic bacteria *M. odoratus* against *P. syntaractis* in lab condition using three different concentrations i.e  $2.6 \times 10^8$  CFU/ml,  $2.6 \times 10^6$  CFU/ml and  $2.6 \times 10^4$  CFU/ml revealed that after 24 hrs of treatment the larvae fed on leaves treated with bacterial suspensions became pale in colour. Subsequently, larval feeding was arrested and change of coloration spread to the entire body surface, and the larvae died. Though feeding of larvae in the treated leaves was not affected in the first 24 hrs, it drastically reduced at 48 hrs with significant loss in body weight and larval mortality observed in all the cases as compared to control (Fig 4 D). The larvae of *P. syntaractis* showed 100 percent mortality in the 48 hrs in the  $2.6 \times 10^8$  CFU/ml, whereas the concentration  $2.6 \times 10^6$  CFU/ml showed 70 % larval mortality. No further studies were carried out by using this bacteria in the field

apart from pathogenicity test on the targeted pest, as there were several reports regarding the bacterial pathogen causing infection to the human beings. Infection of this bacteria on *P. syntaractis* is recorded for the first time. Vivek et al. (2019) reported *M. odoratus* infection in the central nervous system of human beings. Deepa et al. (2014) reported *M. odoratus* and *Chryseobacterium indologenes* bacterium causing infection to human beings.

Utility of pathogenic bacteria in biological control has been well documented over the years (West et al., 1989; Deshmuck & Mathai, 1991). In particular, the use of bacteria in control of forest insect pests has been undertaken in Canada and USA ( Cadogan et al., 1986; Cadogan 1993). Ricardo et al. (2000) reports that different strains of *Bacillus thuringiensis* were found more pathogenic to *Spodoptera frugiperda* causing 80.40 to 100%



larval mortality. Javaregowda and Krishnanaik (2008) reported *B. thuringiensis* as promising when compared to HpNPV and *B. bassiana* in controlling the larvae of *Hyblaea puera*. Similar type of studies were carried out by using native pathogenic bacteria *Bacillus* sp.

at the concentration  $2.6 \times 10^8$  CFU/ml on the bamboo leaf rollers *Psara licarsisalis*, *Pyrausta coclesalis* and *Crocidophora* sp. and found effective in controlling the pests (Raja Rishi et al., 2012).



**Fig. 4:** (A) Larva of *Ptyomaxia syntaractis* on *A. marina* (B) Adult moth *P.syntaractis* (C) Adult moth *P. syntaractis* (enlarged) (D) Effect of entomopathogenic bacteria *Myroides odoratus* against *P. syntaractis*

### CONCLUSION

A growing concern about the impact of chemical insecticides on the environment and development of resistance in insects against currently used chemical pesticides provides incentive for the shift towards use of biopesticides. The pathogenicity tested against the targeted insect pest with the native entomopathogenic bacteria *M. Odoratus*, proved that the bacteria as an ineffective pathogen and is the first time report of this bacteria on the host *P. syntaractis*. The bacterial strain which is specific to a single

species or to a few species, will be more preferable and safer in any biocontrol programme. As per the literature, this pathogen reported is causing infection to the human beings. Therefore further investigation on its effectiveness and cross infectivity are required to confirm any strain variations and the specificity of infection in insects.

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