Endophytic colonization of entomopathogenic *Lecanicillium lecanii* (Zimm) Zare & Gams PTN 10, and its effect on tobacco resistance against *Myzus persicae* Sulzer (Hemiptera: Aphididae)

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Abstract. *Lecanicillium lecanii* is a potential effective entomopathogen against various insect pests. Some isolates of *Lecanicillium* is also reported as endophytic. However, the information on endophytic colonization of *L. lecanii* PTN 10 and its role in conferring tobacco resistance against *Myzus persicae* is not available. The objective of the research was to determine the endophytic colonization ability of *L. lecanii* on tobacco plants and its effect on the biology and population growth of *Myzus persicae*. This research consisted of four treatments i.e. untreated plants (K), seed treated with conidia suspension of *L. lecanii* for 12 hours (P1), foliar spray using conidia suspension *L. lecanii* one week after transplanting (P2), and the combination of seed treatments and foliar spray. All treated tobacco plants were infested by first nymph of *M. persicae* one week after *L. lecanii* treatment. The observations include aspects of biology i.e. life cycle, fecundity, and the longevity of *M. persicae*. *L. lecanii* is proven able to colonize tobacco leaves endophytically. The fungus treatment on tobacco plants causes a prolonged life cycle, decreases fecundity, and significantly shortens longevity. Additionally, it suppresses population growth of *M persicae*.

Keywords: fecundity, life cycle, population growth

1. Introduction

Pest control activities have developed over time. The use of synthetic chemicals for pest control has shifted to a more environmentally friendly, such as biological control methods [1]. Biological control of insects is the use of organisms such as natural enemies or entomopathogens that can kill and cause disease epidemics in the target pest [2]. One of the most widely used entomopathogens for control is *Lecanicillium lecanii*.

The fungus *L. lecanii* is one of the entomopathogens. Some isolates of *L. lecanii* were endophytic, microorganisms that present inside plant tissues without causing any visible symptoms in the host [3]. Endophytic fungi can be found in the environment, and some have been shown to have adverse effects on insects, nematodes, and plant pathogens [3]. Isolate of *L. lecanii* PTN 10 is originated from infected whitefly on tobacco plants. However, there is no information on the endophytic colonization ability of *L. lecanii* PTN 10 on tobacco plants. This knowledge is important to determine appropriate application techniques and to develop *L. lecanii* PTN 10 as a biocontrol agent.

According to [4], isolates of certain entomopathogenic fungi can live as endophytes. Some of these fungi include *Beauveria bassiana*, *Metarhizium anisopliae*, and *L. lecanii*. Other isolates of *L. lecanii*
studied in Australia were reported to be able to colonize cotton leaves and cause a significant decrease in the reproductive rate of *Aphis gossypii* [5]. Other benefits that endophytes provide to their hosts include an increased and sustained plant growth through inhibition of pathogenic microorganisms and invertebrate pests, removal of soil contaminants, increased tolerance to low fertility soils, and increased tolerance to extreme temperatures and low water availability [6]. The research objectives were to assess endophytic colonization of *L. lecanii* PTN 10 and its effect on tobacco resistance against *M. persicae*.

2. Methods

2.1 Experimental design

The research design was a complete randomized design (CRD). The treatments consisted of four treatments; untreated (K), seeds treatments (P1), spraying *L. lecanii* conidial suspension on to tobacco plant one week after transplanting (P2), and a combination of P1 and P2 treatment (P3). Ten replicates, with one plant for each replicate, were used for each treatment for life cycle and periods of stages, fecundity, and longevity of aphids and five replicates for population observations.

2.2 Insect rearing

Wingless adults of aphids/Aptera were taken from tobacco farming sites in Sukaresmi Village, Cianjur Regency, West Java. The aphids were then reared on tobacco plants placed in cages.

2.3 Culturing *L. lecanii* PTN 10

The isolates of the *L. lecanii* PTN 10 were obtained from infected *Bemisia tabaci*, then cultured on PDA pH 5.5 medium and incubated at room temperature for 21 days.

2.4 Inoculation of *L. lecanii* PTN 10

2.4.1 Seed treatment. Seeds of tobacco were wrapped in fairly tight cloth and put in a water bath for 15 minutes at 45°C for hot water treatment (HWT). The seeds were then placed in a petri dish and soaked into a conidial suspension at a rate of $10^5$ conidia ml$^{-1}$, incubated for 12 hours, and sown in trays. The growing medium used was a mix of sterilized soil and manure in a ratio of 1:1.

2.4.2 Spraying application. The tobacco plants were inoculated by spraying their stems and leaves with conidial suspension *L. lecanii* at a rate of $10^5$ conidia ml$^{-1}$ one week after transplanting. Spraying was carried out in the afternoon between 16.30-17.00 WIB. The inoculated plants were then covered by polyethylene bags to maintain the moisture and also to allow the inoculated fungi to penetrate their host plants [7].

2.5 Aphid infestation

Aphid infestation was carried out a week after *Lecanicillium* inoculation. *M. persicae* infested were 1$^\text{st}$ instar and 4$^\text{th}$ instar nymphs for biology and population observations, respectively. Each tobacco plant was infested with 5 aphids for population observation and 1 aphid per plant for biology observations.

2.6 Biological observations of *M. persicae*

2.6.1 Life cycle and periods of stage. Tobacco plants that were 44 days old were infested with 1$^\text{st}$ instar nymphs of *M. persicae* and then covered with mica plastic cages. Observations were made every day starting on the day of infestation until *M. persicae* became imago and produced 1$^\text{st}$ instar nymphs again.

2.6.2 Fecundity and longevity. *M. persicae* that had completed the observed life cycle was reared until the imago *M. persicae* dies to obtain data on the fecundity and longevity of the imago. Newborn nymphs were counted and then killed. The fecundity and longevity were observed every day until the adults died.
2.7 Observations on population growth of *M. persicae*
Observation of the aphid population was carried out by counting the population every day for 30 days using a hand counter.

2.8 Colonization of *L. lecanii* on various parts of plants
Tobacco plants that had been inoculated with fungi were isolated again to verify the presence of fungal colonization. Tobacco plants for each treatment were washed with running water and then air-dried. The leaves, stems, and roots of the tobacco plants were cut into pieces the size of 1 cm x 1 cm. The surface of plant pieces was sterilized twice, using 70% alcohol and 1% NaOCl for 1 minute. The pieces were then washed with sterile water for 1 minute and dried on sterile tissue. The pieces of leaves, stems, and roots were planted on PDA media using tweezers and incubated for 1 week. Reisolation was carried out aseptically in laminar airflow.

2.9 Colonization of *L. lecanii* on tobacco leaves
The middle leaves of all ten replicates per treatment were taken and cut into pieces size of 0.5 cm x 0.5 cm. The surface of ten pieces per leaf per replicate was then sterilized twice as above, then placed onto PDA medium, and incubated for 5 days. A total amount of 10 leaves were taken from each treatment. The colonization frequency was determined by calculating the percentage of leaf cut grown by *L. lecanii* [8].

2.10 Data analysis
The data obtained were analyzed by analysis of variance using the Statistical Analysis System (SAS) program. Significantly different treatments results were further tested using Duncan’s test with a 5% confidence.

3. Results and discussion
3.1 Colonization of *L. lecanii*
Reisolation of the fungus in all treatments (P1, P2, and P3) showed that the fungus *L. lecanii* PTN 10 is able to colonize leaves, but not root and stem (Table 1). Leaves colonization by *L. lecanii* is proven by the growth of *L. lecanii* on leaf cuttings. According to [9], the growth and development of endophytic microorganisms in plant hosts can be systemic or local. Most of the endophytic microorganisms have been documented as nonsystemic [10].

According to [8], *L. lecanii* was reported to be able to colonize cotton leaves previously inoculated with fungi in the laboratory experiment. In addition, this fungus is also endophytic to the families Araceae [11] and *Carpinus caroliniana* [12]. This fungus has also been successfully introduced as an endophyte to date palm (*Phoenix dactylifera*) [13] and cucumber (*Cucumis sativus*) [14].

| Number | Treatment | Colonization of *L. lecanii* |
|--------|-----------|----------------------------|
|        | Root      | Stem | Leave |
| 1      | K         | -    | -     |
| 2      | P1        | -    | -     | +    |
| 3      | P2        | -    | -     | +    |
| 4      | P3        | -    | -     | +    |

(+) : Colonized ; (-) : Not colonized

Colonization of *L. lecanii* PTN 10 at the four treatments showed different rates. The highest frequency of colonization was in P3 with an average value of 54%, while P1 and P2 were 46% and 40%, respectively. *L. lecanii* PTN 10 was not found in the untreated leaves (Table 2). The most important treatment for fungal colonization was the application of *L. lecanii* PTN 10 suspension to the
seeds while spraying slightly increased the frequency of colonization (Table 2).

Table 2. The frequency of colonization of *Lecanicillium lecanii* PTN 10 on tobacco leaf.

| No | Treatment | Colonization frequency (%) |
|----|-----------|-----------------------------|
| 1  | K         | 0 a                         |
| 2  | P1        | 46 b                        |
| 3  | P2        | 40 b                        |
| 4  | P3        | 54 b                        |

a Numbers followed by the same letter in the same column are not significantly different based on Duncan’s test at 5% level.

3.2 The life cycle of *M. persicae*

Aphids *M. persicae* reproduces by parthenogenesis. The nymph stage is about 6 days [15]. Aphids undergo four instars to become imago with an average of 2 days. The average daily reproduction is 1.6 nymphs per female [16].

The research showed that the longest life cycle among other treatments was P2. The shorter life cycle of the aphids in the control caused the aphids to produce nymphs faster. Generally, tobacco treated by *L. lecanii* PTN 10 has longer life cycles of aphids. This can affect the population development of the aphids (Table 3).

The magnitude of the effect of the fungus *L. lecanii* application on the biology and population of aphids is related to the level of colonization. The more fungi that colonize the leaves, the greater the effect on the biology and population of the aphids. According to [17], most endophytic microbes do not directly contact plant pest organisms. Endophytes play an indirect role in biological control.

3.3 Fecundity of *M. persicae*

The total fecundity of *M. persicae* in the fungus inoculated plants was significantly lower than untreated (Table 4). This could be due to the different average lifespan of aphids in each treatment, so that it affected the number of nymphs produced. The longer the age of the aphids imago, the more time the aphids can produce nymphs so that the population can increase and vice versa. In addition, the level of colonization of the entomopathogenic *L. lecanii* PTN 10 in the treated plants also affected the aphids fecundity. The higher the frequency of colonization of *L. lecanii* on tobacco leaves, the more *L. lecanii* affects the biology of aphids. [18] argues that many endophytic microorganisms become biological agents with an antibiosis mechanism. Many endophytic bacteria and fungi produce secondary metabolites that have antagonistic traits. Aphids can die due to metabolites produced by fungi that colonize the plants.

3.4 Population growth of *M. persicae*

Environmental conditions such as temperature are one of the most influential factors on aphid populations [19]. Other factors are the cultivation system [20], plant varieties [21], and the use of chemicals. Intensive use of synthetic insecticide can cause loss of natural enemies and increase the population of aphids.

The research showed that fungal treatment on tobacco plants could affect the infested *M. persicae* population. The population of aphids on the treatment P1, P2, and P3 is lower compared to the control. The peak of population increase generally occurred on day 24 to day 28 and experienced a significant decrease after infestation on days 29 and 30. On the 30th day after infestation, the aphid population in P1 and P3 treatments was significantly different from the control (Figure 1).
Figure 1. Population growth of *Myzus persicae* on inoculated tobacco plants.

According to [5], endophyte, which occupies the entomopathogenic plant tissue, can potentially interact with insect pests in various ways. Endophyte can produce conidia on leaf surfaces, which can be in contact with the insect. Insects can also contact fungal metabolites through ingestion of the leaf juice.

**Table 3. *Myzus persicae* life cycle in tobacco plants treated by *Lecanicillium lecanii* PTN 10.**

| No | Treatment | Periods (days) | Instar | Nymph body length (mm) | Pre-childbirth Period | Life Cycle (days) |
|----|-----------|----------------|--------|------------------------|-----------------------|-------------------|
|    |           | 1              | 2      | 3                      | 4                     |                   |
| 1  | K         | 2.10b          | 1.10b  | 1.70a                  | 1.40a                 | 6.20 ± 0.78b      | 1.30 ± 0.48ab     | 7.60 ± 0.84b      |
| 2  | P1        | 2.20b          | 1.50ab | 1.60a                  | 1.60a                 | 6.90 ± 0.87ab     | 1.00 ± 0b         | 8.00 ± 0.82ab     |
| 3  | P2        | 2.40ab         | 1.70a  | 1.90a                  | 1.40a                 | 7.30 ± 1.25a      | 1.60 ± 0.70a      | 8.90 ± 1.59a      |
| 4  | P3        | 2.70a          | 1.50ab | 1.70a                  | 1.50a                 | 7.40 ± 0.96a      | 1.00 ± 0b         | 8.30 ± 0.48ab     |

a Numbers followed by the same letter in the same column are not significantly different based on Duncan’s test at 5% level.

**Table 4. Fecundity of *Myzus persicae* in tobacco plants treated by *Lecanicillium lecanii* PTN 10.**

| No | Treatment | Amount of progeny in day.... |
|----|-----------|-----------------------------|
|    |           | 1              | 2      | 3                      | 4                      | 5                      | 6                      | 7                      | 8                      |
| 1  | K         | 2.60 ± 0.69a 3.00 ± 0.94a | 2.50 ± 1.08a | 2.80 ± 1.03ab | 2.00 ± 1.63a | 3.40 ± 1.77a | 1.60 ± 0.96b | 1.50 ± 1.08b |
| 2  | P1        | 1.80 ± 0.78a 2.00 ± 1.49a | 2.50 ± 1.35a | 2.80 ± 1.87ab | 2.00 ± 0.94a | 2.10 ± 1.44ab | 1.75 ± 1.28b | 1.00 ± 0.81b |
| 3  | P2        | 2.10 ± 1.10a 2.80 ± 1.39a | 2.40 ± 1.34a | 3.00 ± 1.56a | 3.00 ± 1.65a | 1.88 ± 1.26b | 3.33 ± 1.41a | 3.25 ± 1.28a |
| 4  | P3        | 2.40 ± 1.26a 1.90 ± 0.99a | 1.20 ± 1.22a | 1.60 ± 0.96b | 1.80 ± 0.78a | 1.80 ± 1.61b | 1.40 ± 1.14b | 2.33 ± 2.08ab |

a Numbers followed by the same letter in the same column are not significantly different based on Duncan’s test at 5% level.
Table 4 (Continued) Fecundity of *Myzus persicae* in tobacco plants treated by *Lecanicillium lecanii* PTN 10 for 13 days on tobacco plants.

| No | Treatment | Day | Total fecundity | Longevity (days) |
|----|-----------|-----|-----------------|-----------------|
| 1  | K         | 9   | 2.66 ± 2.00a    | 2.50 ± 1.29a    | 3.33 ± 1.52a    | 10.00 ± 1.76a |
|    |           | 10  | 3.00 ± 1.87a    | 5.00 ± 2.00a    | 3.33 ± 1.52a    | 10.00 ± 1.76a |
|    |           | 11  | 2.50 ± 1.29a    | 5.00 ± 2.00a    | 3.33 ± 1.52a    | 10.00 ± 1.76a |
|    |           | 12  | 3.00 ± 1.52a    | 5.00 ± 2.00a    | 3.33 ± 1.52a    | 10.00 ± 1.76a |
|    |           | 13  | 2.50 ± 1.29a    | 5.00 ± 2.00a    | 3.33 ± 1.52a    | 10.00 ± 1.76a |
| 2  | P1        | 9   | 2.00 ± 1.15a    | 2.50 ± 1.91a    | 3.00 ± 0.57a    | 14.00 ± 7.923b |
|    |           | 10  | 2.50 ± 1.91a    | 3.33 ± 0.57a    | 3.00 ± 0.57a    | 14.00 ± 7.923b |
|    |           | 11  | 3.00 ± 0.57a    | 3.00 ± 0.57a    | 3.00 ± 0.57a    | 14.00 ± 7.923b |
|    |           | 12  | 3.00 ± 0.57a    | 3.00 ± 0.57a    | 3.00 ± 0.57a    | 14.00 ± 7.923b |
|    |           | 13  | 3.00 ± 0.57a    | 3.00 ± 0.57a    | 3.00 ± 0.57a    | 14.00 ± 7.923b |
| 3  | P2        | 9   | 2.60 ± 1.81a    | 3.00 ± 0.0a     | -               | 22.70 ± 7.10ab |
|    |           | 10  | 2.66 ± 2.08a    | 3.00 ± 0.0a     | -               | 22.70 ± 7.10ab |
|    |           | 11  | 3.00 ± 0.0a     | 3.00 ± 0.0a     | -               | 22.70 ± 7.10ab |
|    |           | 12  | 3.00 ± 0.0a     | 3.00 ± 0.0a     | -               | 22.70 ± 7.10ab |
|    |           | 13  | 3.00 ± 0.0a     | 3.00 ± 0.0a     | -               | 22.70 ± 7.10ab |
| 4  | P3        | 9   | -               | -               | -               | 12.10 ± 3.87c  |
|    |           | 10  | -               | -               | -               | 12.10 ± 3.87c  |
|    |           | 11  | -               | -               | -               | 12.10 ± 3.87c  |
|    |           | 12  | -               | -               | -               | 12.10 ± 3.87c  |
|    |           | 13  | -               | -               | -               | 12.10 ± 3.87c  |

a Numbers followed by the same letter in the same column are not significantly different based on Duncan’s test at 5% level

4. Conclusion

*L. lecanii* PTN 10 is able to colonize endophytically in the tobacco leaves. Treatment of the funguson tobacco confers tobacco resistance against *M. persicae* indicated by prolonged cycle, decreased fecundity, decreased longevity, and suppressed population growth.

References

[1] Shahid AA, Rao AQ, Bakhsh A and Husnain T 2012 *Arch Biol Sci.* 64 21–42
[2] Vu VH, Hong S I and Kim K 2007 *J Biosci Bioeng.* 104 498–505
[3] Vega FE, Posada F, Aime MC, Pava-ripoll M, Infante F and Rehner SA 2008 *Biol Control.* 46 72–82
[4] Vidal S and Jaber LR 2015 *Curr Sci.* 109 46–54
[5] Gurulingappa P, Mcgee PA and Sword G 2011 *Crop Prot.* 30 349–53
[6] Card S, Johnson L, Teasdale S and Caradus J 2016 *FEMS Microbiol Ecol* 88 1–44
[7] Hermawati H 2007 [skripsi] Bogor (ID): Institut Pertanian Bogor
[8] Anderson CM, McGee PA, Nehr DB and Mensah RK 2007 *Australas Mycol.* 26 65–70
[9] Boyle C, Gotz M, Damman TU and Schulz B 2001 *Symbiosis.* 31 259–81
[10] Canals RM, San-emeterio L, Sanchez-Marquez S, de los Mozos IR, Pujol P and Zaloglobuezacoal 2014 *Spanish J Agric Res.* 12 623–32
[11] Petriti O and Dreyfuss M 1981 *Microb Inst.* 135 48–51
[12] Bills GF and Polishook JD 1991 *Can J Bot.* 69 1477–82
[13] Gómez-Vidal S, Lopez-Liorca LV, Jansson HB and Salinas J 2006 *Micron.* 37 624–32
[14] Benhamou N and Brodeur J 2001 *J Mol Plant Pathol.* 58 133–44
[15] Kalschoven LGE 1981 *The pest of crops in Indonesia* Laan PA van der, translator Jakarta: PT Ichtiar Baru-van Hoeve, translated from: De Plagen van de Cultuurgewassen
[16] Capineria JL 2001 *University of Florida Institute Food and Agricultural Sciences.* 1-9
[17] Danielsson J, Reva O and Meijer J 2006 *Microb Ecol.* 54 134–40
[18] Yan JF, Broughton SJ, Yang SL, Gange AC and Jnr JW 2014 *Fungal Ecol.* 13 53–9
[19] Davis JA, Radcliff EB and Ragsdai DW 2006 *Environ Entomol.* 35 1461–68
[20] Siddauruk L, Bakti D, Kuswardan RA and Hanum C 2015 *Int J Sci Technol Res.* 4 272–7
[21] Mdellel L and Kame MBH 2014 *Eur J Environmental Sci.* 4 102–05