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Pseudomonas fluorescens Induced resistance against *Liriomyza trifolii* (Burgess) on parthenocarpic cucumber

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Abstract
Induced resistance refers to the induced state of plants triggered by chemical or biological inducers, which in turn protects other non-exposed plant parts against the expected future attack by herbivorous insects. Any compound that is used to trigger the induced response of the plant is called elicitors. The idea is to keep the plant ready for the pest attack before it is actually exposed to the herbivores damage. Serpentine leaf miner *Liriomyza trifolii* (Burgess) is one of the major pests of cucumber under greenhouse condition. In the current study *Pseudomonas fluorescens* is tried as a elicitor in order to trigger the induced response of the cucumber plants against the leafminers. *Pseudomonas fluorescens* IOF 1 strain was sprayed on the parthenocarpic cucumber plants that were grown under greenhouse conditions at the rate of 5g/ litre. Spray was given before the beginning of actual attack of the leafminers just after twelve days after the opening of cotyledon leaves. It was found that the average number of leafminings recorded on the *P. fluorescens* treated plants were lesser (0.79 mines/ leaf) than the untreated control plot (1.99 mines/ leaf). And also up to three weeks *P. fluorescens* sprayed cucumber plants were free from any leafminers. Brief explanation of the present study is explained in this paper.

Keywords: induced resistance, *Liriomyza trifolii*, *Pseudomonas fluorescens*

Introduction
Plants and insects were evolved together and living in harmony with each other for millions of years. In co- evolution, both have acquired different strategies to avoid and break each other's defense systems whenever it was necessary. Herbivores attacks been responded by plants through complex and efficient defense system that includes toxic chemicals, structural barriers and attraction of natural enemies of the target pests (Howe and Jander, 2008, Hanley *et al*., 2007) [7, 6]. Direct and indirect defensive mechanisms might present as a part of the physical structure or induced after damage by the herbivores. Induced resistance refers to the induced state of response of plants triggered by chemical or biological inducers, which in turn protects other non exposed plant parts against the expected future attack by herbivorous insects (Kuc, 1982) [8]. Dhaliwal *et al*., 2013 [3] defines the same as the qualitative enhancement of a plants defense mechanisms against pests in response to external physical or chemical stimuli results in change in a plant that produce a negative effect on herbivores. This is a non-heritable resistance where host plants are induced to impart resistance to tide over pest infestation. (Dhaliwal *et al*., 2013) [3]. Any compound that is used to trigger the induced response of the plant is called as elicitors. It can be anything like larval oral secretion, plant growth promoting bacteria, phytohormones etc (Hemm *et al*., 2010 Meenakshi and Baldev, 2013) [5, 10]. Serpentine leaf miner *Liriomyza trifolii* (Burgess) is one of the major pests of cucumber under greenhouse condition all around the world. *Pseudomonas fluorescens* is mostly known for its ability to be used as biocontrol agents against the diseases (Laha *et al*., 1992; Ganeshan, G and Kumar, 2005, Vanitha and Ramjegathesh, 2014) [9, 4, 14] but there are studies proving its other side of using them as successful insect pest biological control agent too by its growth promoting activity or inducing the secondary metabolites in the plant. When the cucumber plants were treated with *Pseudomonas fluorescens* it attracted lesser number of spider mites. When the mites were introduced on the treated plants and recorded six weeks after infestation the density of the mite population was 2-fold lower on bacteria-treated cucumber comparing with the untreated control plot (Anna, 2006) [1]. While the talc-based formulation of *Pseudomonas fluorescens* and *Beauveria bassiana* was sprayed against seedling blight in groundnut under glasshouse conditions it also controlled the groundnut leafminer (*Aproaerema modicella*) (Senthilraja *et al*., 2010) [12]. Meca *et al*. (2009) reported 70 per cent mortality of citrus leaf miner larvae, *Phyllocnitis citrella* under laboratory condition when it was treated with *Pseudomonas sp*.

Usually spray for plant protection measures starts right after the pest or symptoms appearance. But what if we spray before the pest infestation and make them defend themselves when the pest attacks. This idea of inducing the resistance in plants before the attack of the herbivore is evaluated in the present study using the external spraying of *Pseudomonas fluorescens* on the cucumber plants as follows,

Materials and Methods

Research trial has been carried out under greenhouse condition in Hi-tech horticulture unit, University of Agricultural Sciences, Dharwad. The study was repeated for two seasons in summer (March to June) and winter (August to November). Parthenocarpic cucumber variety KPCH-1 was taken for the study which was collected from Kerala Agriculture University, Trissur. *Pseudomonas fluorescens* IOF 1 strain was purchased from Institute of Organic forming, UAS, DWD. Research block was divided into ten plots (1 m (width) x 12 m (length)) and every alternated plot was sprayed with *Pseudomonas fluorescens* IOF 1 strain @ 5g/l leaving five untreated plots (control) and five treated plots. Three such sprays were given in ten days interval from twelve days after the opening of cotyledonous leaves.

Observation

The number of live mines on five randomly selected leaves per plant is counted and recorded as recommended by Anon, 2014 [2] from the leafminers (*Liriomyza trifolii*) appearance till the last harvesting.

Statistical analysis

Data was transferred using square root transformation before going into the analysis. Student's t test has been used to analyze to find if there is any variation found between the treated and control plots. When the calculated t was more than the table T it was taken as significant difference between the treatments.

Results

Results of the two season studies are presented in the Table 1. Based on the data the variation between the *Pseudomonas fluorescens* treated plot and the untreated control plot is clear as the calculated t is greater than the table T in both the seasons. Average number of leaf mining located per leaf on the *P. fluorescens* treated plants was 0.79 were as it was 1.99 in case of the plants in the control plots. It can also be noticed that the *Liriomyza trifolii* did not prefer to lay the eggs on the pseudomonas treated cucumber leaves initially up to two weeks during both the seasons. Afterwards there was some population build up in the treated plot which was less than half of the leaf mining found on the untreated control plot which is obvious cause of the induced resistance which was triggered well before the pest infestation. Towards the end of the season the *P. fluorescens* sprayed plants also preferred by the leafminers reaching the maximum population of 2.34 mines per leaf during summer and 3.56 mines per leaf during winter. While comparing with the control plot without any treatment recorded 3.05 mines, 4.68 mines per leaf during summer and winter respectively and this is still more than the treated plot. In case of *P. fluorescens* sprayed plots it was only after the second week where the leafminers slowly started to appear (0.05 mines/ leaf) and reaching up to 2.95 mines per leaf towards the end of the season.

Table 1: Leafminer (*Liriomyza trifolii*) damage on *Pseudomonas flurosence* treated and untreated leaf

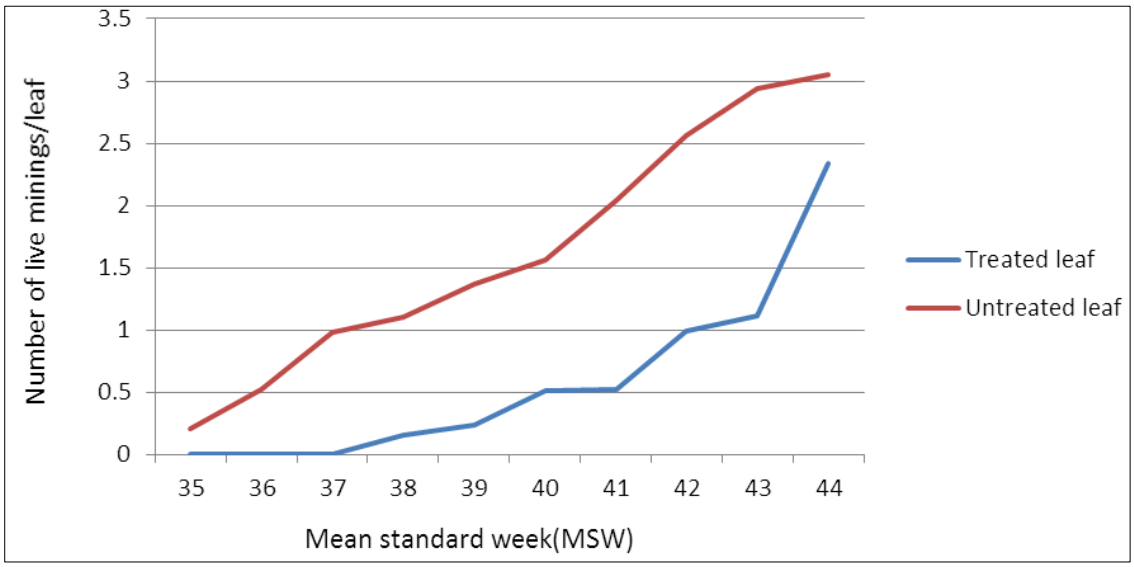
Date of observation (MSD)	No of live mining's per leaf		Date of observation (MSD)	No of live mining's per leaf		No of live mining's per leaf	
	Summer			Winter		Pooled	
	Treated	Control		Treated	Control	Treated	Control
14	0.00 (0.71)	0.21 (0.84)	35	0.00 (0.71)	0.24 (0.86)	0.00 (0.71)	0.23 (0.85)
15	0.00 (0.71)	0.52 (1.01)	36	0.00 (0.71)	0.86 (1.17)	0.00 (0.71)	0.69 (1.09)
16	0.00 (0.71)	0.98 (1.22)	37	0.10 (0.77)	1.15 (1.28)	0.05 (0.74)	1.07 (1.25)
17	0.15 (0.81)	1.10 (1.26)	38	0.28 (0.88)	1.96 (1.57)	0.22 (0.85)	1.53 (1.42)
18	0.24 (0.86)	1.37 (1.37)	39	0.54 (1.02)	2.10 (1.61)	0.39 (0.94)	1.74 (1.49)
19	0.51 (1.00)	1.56 (1.44)	40	0.78 (1.13)	2.26 (1.66)	0.65 (1.07)	1.91 (1.55)
20	0.52 (1.01)	2.04 (1.59)	41	0.96 (1.21)	2.98 (1.87)	0.74 (1.11)	2.51 (1.73)
21	0.99 (1.22)	2.56 (1.75)	42	1.26 (1.33)	3.35 (1.96)	1.13 (1.27)	2.96 (1.86)
22	1.11 (1.27)	2.94 (1.85)	43	2.51 (1.73)	3.89 (2.10)	1.81 (1.52)	3.42 (1.98)
23	2.34 (1.69)	3.05 (1.88)	44	3.56 (2.01)	4.68 (2.28)	2.95 (1.86)	3.87 (2.09)
MEAN	0.59	1.63		1.00	2.35	0.79	1.99
Calculated T	2.83			2.33		2.48	
Table t	2.10			2.15		2.12	

*Values in the parenthesis are in square root+0.5 transformations, *MSD- Meteorological standard week

Fig 1: Effect of induced resistance due to *P. fluorescens* treatment

Discussion

Plants those were sprayed with *Pseudomonas fluorescens* did not attract or free from the leafminings for fifteen days while the untreated plants were damaged with the leafminings. Initially the plants treated with *P. fluorescens* have shown the induced response which repelled the leafminers away from them and those adults were settled on the untreated plants which doesn't produce any induced response and started to replicate on them. When the time proceeds, as the induced response started to decline in the treated plants, the leafminers stated to lay their eggs and started to mine the leaves. Present findings are in line with Senthilraja *et al*., 2010 [12] who also came across the controlled activity of groundnut leafminer (*Aproaerema modicella*) when it was actually sprayed to control the seedling blight of groundnut. Similar kind of result was obtained when Anna, 2006 [1] treated the cucumber leaves with *P. fluorescens* which ultimately reduced the incidence of two spotted spider mites. As *P. fluorescens* does not have any direct insecticidal properties it is safe to say that the phytochemicals that was triggered by spraying *P. fluorescens* on the plant created such a reduced incidence of the leafminers. It can also be linked with the other growth promoting bacterias which were also responsible for creating herbivore mediated induced response in plants by inducing the production of defensive compounds as reported by van de Mortel *et al*., 2012 [13]; Pangesti *et al*., 2016 [11]. Response of the cucumber plants to the leafminer population due to the external application of *P.fluorescens* could be related with the treatment with *Bacillus* spp which produced the JA induced pathway towards the induced response which produced the control over the spodoptera caterpillars as reported by Wu *et al*., 2010 [15] and Zebelo *et al*., 2016 [16]. These are all the possible ways that *P. fluorescens* could have created on the cucumber plant which was unfavourable to the multiplication of Liriomyza *trifolii* during the initial phase of the crop.



Graph 1: Effect of induced resistance on *L. trifolii* population

Conclusion

It was noticed that the cucumber plants that was sprayed with *Pseudomonas fluorescens* IOF 1 had lesser number of *Liriomyza trifolii* damage during the research period. Although the lesser population of leafminers can be correlated with the induced response of the cucumber plants based on the previous studies, it is also necessary to identify the exact phytochemical and pathway it is produced in order to take this study to the next level in constructing the natural defense system against the pests before the expected possible attack. By identifying and spraying the elicitors of induced responses on the plants, it is highly possible to construct the natural defense system against herbivores damage. In future studies should be carried out to find the molecular level reason behind the induced resistance.

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