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In vitro Estimation on the Effect of Imidacloprid on the Growth and Multiplication Rate of *Rhizobium* for a Strategic-Ecofriendly Seed Treatment in Pulse

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A B S T R A C T

In Pulses particularly in blackgram and greengram seed treatment, Imidacloprid is one of the important component used for the control of viral diseases particularly Yellow Mosaic disease, in addition, biofertilizers *viz.*, *Rhizobium* and phosphobacteria also necessarily used to promote nodulation and nitrogen fixation. These field recommendations should be well supported by *in vitro* assays and testing the compatibility of the pesticides with the biofertilizers and biopesticides used for seed treatment. To confirm the compatibility an *in vitro* study was conducted on the effect of Imidacloprid dosage on the growth and multiplication using YEMA broth and the growth rate was enumerated in Spectrophotometer. Imidacloprid did not prevent the growth of *Rhizobium* in the growth media rather Imidacloprid showed a retarded effect towards *Rhizobium* hence, the growth of *Rhizobium* was initially set back and registered very slow growth rate upto 1½ days after that it attained normal growth and reached its log phase.

Introduction

Pulses play a vital component in our food, because of its protein content (24-26%) which not only vitalize human being but soil also by nourishing with nitrogen it enrich the soil fertility. Among pulses, blackgram and greengram occupies more area over total pulse production. Blackgram is one of the most important pulse crop normally grown in Tamilnadu. Black gram (*Vigna mungo* (L.) Hepper) is the third important pulse crop in India. (Anonymous, 2010) which is a short-duration *Kharif* grain legume belongs to the family Fabaceae and is a rich source of protein which is one of the essential nutrients of human. Black gram contributes 11% to the national pulse production from an area of 14%. Blackgram is affected by different species of insect pests and diseases. Among the diseases, viral diseases *viz.*, Mungbean Yellow Mosaic Virus (MYMV) and leaf curl viruses which affects the growth and productivity of blackgram. MYMV is the most

critical viral disease which limits the crop productivity in a drastic way because MYMV incidence will quickly spread and even to the seeds also changed its original colour. Hence, the yield reduced and ultimately affects the market value of the seeds. Even these MYMV infected seeds could not be used for next generation also.

Hence, for MYMV management prevention is better than control, or MYMV resistant varieties can be used for cultivation. Among the different methods of MYMV disease management, seed treatment is better, strategic and ecofriendly also. Many improved and integrated techniques are available, to increase the productivity of pulses, among which seed treatment is one of the best integrated method for any crop interms of crop nutrition to bring healthy seedlings and protect the seedling from pests and disease attack even from young initial period of growth. In pulses seed treatment is practiced as combinations with seed protectant pesticides *viz.,* Imidacloprid and biofertilizers and biopesticide (*Pseudomonas fluorescence).* The rhizobial inoculant and phosphate solubilizing bacteria (PSB) are commonly applied to seeds of legume crops to ensure effective nitrogen fixation by *Rhizobium* and solubilisation of native phosphorus by PSB, thereby making the two essential nutrients available to the crop (Gyaneshwar *et al.,* 2002). Hence, in seed treatment, the *Rhizobium* and PSB is directly and immediately exposed to the pesticide. Though it is practiced under field conditions, the compatibility of the pesticides with the biofertilizers and biopesticides was not well documented. Normally pesticides used in seed treatments of pulses should not affect adversely both the seedling and the beneficial microbes used. There are reports which suggest that herbicides may have negative effects on growth of rhizobia (Kyei-Boahen *et al.,* 2001; Revellin *et al.,* 1992) although other reports revealed no such adverse effects (Kunal and Poonam Sharma, 2012; Annapurna *et al.,* 2000). Because insecticide seed treatments are often formulated as combinations with other seed protectant pesticides, biofertilizers and biocontrol agents.

Use of seed treatments in Integrated Pest Management of major field crops has increased considerably over the past few years worldwide (Thube *et al.,* 2016). In general, in black gram and greengram, during seed treatment first Imidacloprid is treated @ 5 ml/kg of seed followed by biofertilizers (*Rhizobium* and phosphobacteria @ 200g/ ac seed) and *Pseudomonas (@* 10g/kg of seed is used. Imidacloprid should not affect the beneficial microbes used, based on this to study the compatibility of Imidacloprid with *Rhizobium,* phosphobacteria and *Pseudomonas* the present investigation was undertaken under laboratory conditions.

Materials and Methods

To study the compatibility of Imidacloprid with *Rhizobium* and phosphobacteria used in seed treatment of blackgram for the control of MYMV an *in vitro* test was conducted. Recommended *Rhizobium* (BMBS 47) and Phosphobacteria (Ps1) were procured from Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore. The *Rhizobium* was initially maintained on Yeast Extract Mannitol broth (YEMA) (Allen and Baldwin, 1931) and allowed to grow upto its log phase (36 hrs). The recommended concentration of Imidacloprid (5ml/kg) was converted to laboratory concentration on the basis of already reported calculations (Fletcher *et al.,* 1956). The log phase *Rhizobium* culture was inoculated @ 1% to YEMA broth added with Imidacloprid. Another YEMA broth (without Imidacloprid addition) was inoculated with the same and incubated at room temperature and

the growth of *rhizobium* was measured in Spectrophotometer at 620 nm from 0 hr. During initial period of growth of *Rhizobium*, OD value was taken at 1 hr intervals (upto 5 hrs), 2 hrs intervals (upto 13 hrs), 3 hrs intervals (upto 25 hrs.), 4 hrs intervals (upto 33 hrs) finally 6 hrs intervals upto 51 hrs and the OD value was tabulated.

Results and Discussion

The results of the experiment revealed during the first 4 hrs of incubation period *Rhizobium* growth rate was observed very slow in the both the medium. After 4 hrs of incubation the growth rate was gradually increased upto 11 hrs of incubation after that the growth rate was increased exponentially (from 0.1 OD to 0.240 OD) in YEMA broth. The growth of *Rhizobium* was observed normal increase in growth and reached its log phase by 16 hrs after inoculation whereas, in Imidacloprid added YEMA broth *Rhizobium* growth was observed very slow and reached its log phase after 33-35 hrs of inoculation.

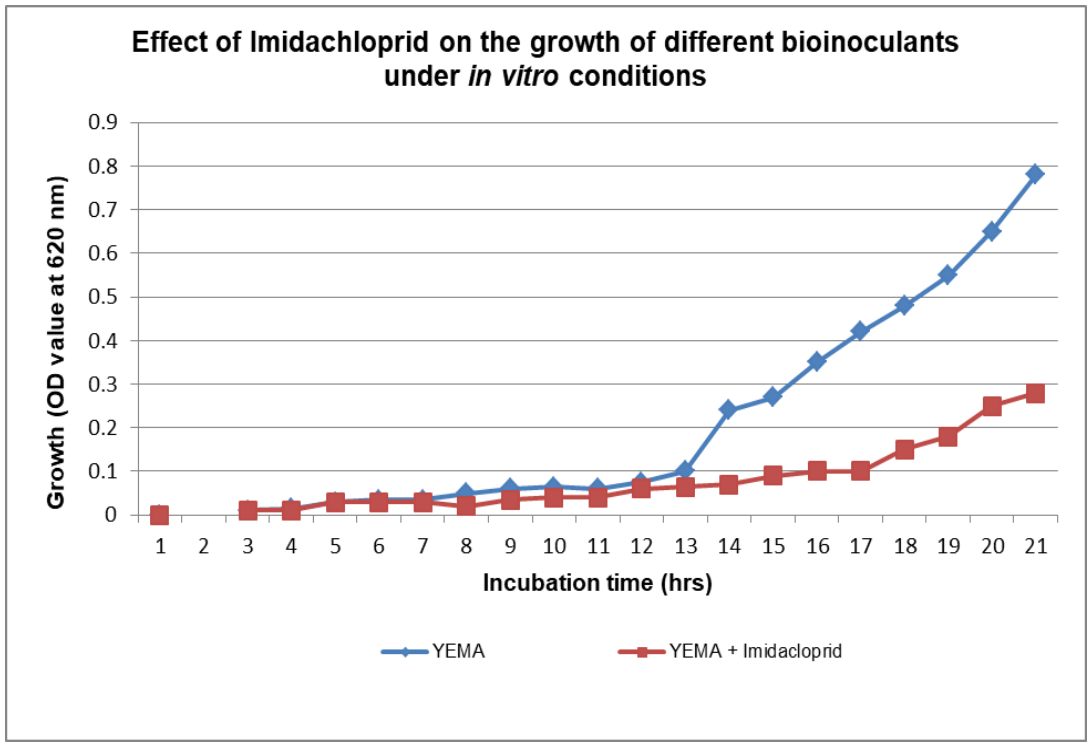
This may be due to the toxic effect of Imidacloprid persisted during initial period the *Rhizobium* was susceptible or it showed delayed growth. Later on (after 35 hrs) the toxic effect of Imidacloprid was slowly subsidized which allowed the growth of *Rhizobium* in later hrs of incubation. Hence, Imidacloprid did not arrest or prevent *Rhizobium* growth rather it retarded the growth for a period of 1 to 1.5 days. The toxic effect of Imidacloprid was gradually reduced when time of incubation extends hence, after 35 hrs of inoculation the growth of *Rhizobium* was observed normal and increased.

Thube *et al.,* (2016) also reported that treatments *viz.,* Imidacloprid @ 5 ml/kg + *Mesorhizobium* @ 25 g/kg of seed Imidacloprid @ 5 ml/kg and Imidacloprid @ 5 ml/kg + *Mesorhizobium* @ 25 g/kg of seed + *Trichoderma* @ 4 g/kg seed was found IPM compatible and eco-friendly seed treatment tactics. The toxicity of Captan (38%) to viable rhizobial cells was higher as compared to Chlorpyrifos (25%) and Endosulfan (30%). The number of viable rhizobial cells was reduced significantly after the initial 4-h contact with fungicide and insecticides and *Mesorhizobium* sp. Cicer and it further decreased during 8-h to 16-h contact (Kyei-Boahen *et al.,* 2001). Similarly, the mixture of Lindane and Chlorpyrifos had no deleterious effect on the survival of B. japonicum after 24-h contact with soybean seed (Revellin *et al.,* 1992). However, a drastic reduction in growth was reported by Tu (1971) and the prolonged storage reduced the number of rhizobia. The rapid loss of rhizobia viability due to the deleterious effect of Captan was reported in Chickpea (Kunal and Sharma, 2012) and in Soybean *B. japonicum* (Annapurna *et al.,* 2000), *R. phaseoli* (Graham *et al.,* 1980) and *R. ciceri* (Kyei-Boahen *et al.,* 2001).

Kunal and Sharma (2012) also concluded that the recommended rates of fungicide and insecticides as seed treatment were not detrimental to chickpea-*Mesorhizobium* sp. Cicer symbiosis, hence they can be safely used to obtain higher productivity. Singh and Wright (2002) also reported in their *in vitro* study that, in broth culture, the herbicides *viz.,* terbutryn/terbuthylazine at 64.0 mg^{-l})1, trietazine/simazine at 68.8 mg^{-l}) and prometryn at 64.0 mg^{-l}) decreased rhizobial growth by only 12.9, 4.7 and 5.9%, respectively, although these rates are 15–20 times higher than the recommended field application rates. He also stated that the herbicides added in yeast mannitol broth did not adversely affect the growth of rhizobia at the concentrations expected to be normally experienced by the rhizobia under field conditions; the growth was affected only at higher concentrations.

Table.1 Colorimetric estimation of *in vitro* sensitivity of *Rhizobium* to Imidacloprid

S. No.	Time Interval (hrs.)	*Rhizobium* growth interms of OD value @ 620 nm	
		YEMA broth	YEMA + Imidacloprid
1	0	0.01	0.01
2	1	0.015	0.01
3	2	0.03	0.03
4	3	0.035	0.03
5	4	0.035	0.03
6	5	0.05	0.02
7	7	0.06	0.035
8	9	0.065	0.04
9	11	0.06	0.04
10	13	0.075	0.06
11	16	0.1	0.065
12	19	0.24	0.07
13	22	0.27	0.09
14	25	0.35	0.1
15	29	0.42	0.1
16	33	0.48	0.15
17	39	0.55	0.18
18	45	0.65	0.25
19	51	0.78	0.28



Therefore, it is possible that herbicides may have little effect on the growth of rhizobia (as observed in the present study) but may still adversely affect the nodulation process and nitrogenase activity.

The herbicides do not affect cell division, the outer wall of the bacterial strain may have been impermeable to these herbicides and the nodulating capacity of the herbicide-treated rhizobia may be decreased (Martensson 1992; Madhavi *et al.*, 1993, 1994).

The Present study also concluded that Imidacloprid did not affect the growth and multiplication rate of *rhizobium* inoculated in the YEMA broth rather it retarded the growth during the initial period. Hence, it is advisable that Imidacloprid or any pesticide treated seeds may be further treated with biofertilzer *Rhizobium* after 1 or 2 days depending upon the toxicity of the pesticides.

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