J. Agr. Sci. Tech. (2011) Vol. 13: 27-33

Effects of Two Systemic Insecticides on Damping-off Pathogens of Cotton

M. Houshyiar Fard[1*], and T. Darvish Mojeni [2]

ABSTRACT

***In vitro* and greenhouse studies were conducted to investigate the possibility of an interaction between two systemic insecticides of: Thiodicarb (Larvin DF 80) and Imidacloprid (Guacho SW 70), and seedling disease organisms of: *Rhizoctonia solani*, *Fusarium moniliforme* and *Pythium ultimum*. When *in vitro* concentrations of Imidacloprid and Thiodicarb were applied, a fungistatic activity occurred. These insecticides inhibited mycelial growth of *R. solani* and *F. moniliforme* by 6.6-14.1 % and 15.2-70.8 %, respectively. *F. moniliforme* was more seriously affected by the insecticides while no significant effect was observed on *P. ultimum.* The pot experiments confirmed *in vitro* results so that, Thiodicarb provided excellent protection against pre- and post-emergence damping-off of the plant by 54.2% and 90.6 %, respectively.**

Keywords: Antifungal activity, *Fusarium moniliforme*, Imidacloprid, *Pythium ultimum*, *Rhizoctonia solani*, Thiodicarb.

[DOR: 20.1001.1.16807073.2011.13.1.1.4]

INTRODUCTION

Damping-off and seedling root rot are serious problems of cotton in cotton producing regions. *Pythium ultimum* Trow, *Rhizoctonia solani* Kűhn and *Fusarium* spp. are the major fungi associated with the diseased cotton plants (Johnson *et al*., 1970; Minton and Garber, 1983; Colyer, 1988). The use of insecticides applied at planting time to control cotton early season pests has been recommended (Hawkins *et al*., 1966; Rummel and Quisenberry, 1979). Thiodicarb [(dimethyl n,n′-thio bis (methylimino) carbonyloxy bis ethan imidothioate)] belongs to the group of carbamates with stomach and contact action properties. This insecticide is of systemic property and is durably persistent in crop plants. It is recommended for control of the lepidoptera, coleoptera, and maggots in cotton and several other crops. Imidacloprid, 1-[(6-chore-Pyridy) methyl]-N-nitro-2-imidazolidiniminee 4- Nitromethylene] belongs to the nitroguanidin group which has been commercially produced as FS 350 and WS 70 formulations. This insecticide has root-systemic property and is used for seed treatment in corn, cotton, sorgum, sunflower, potato, cereals as well as sugar beet against sucking and chewing pests. As is known, agricultural chemicals interact with biological systems in soil so that, a considerable amount of seed treating pesticides persistent in the upper soil layers around the treated seed could exert adverse effects on soil microorganisms. The side effects of pesticides on soil microflora have been studied by several authors (Hector *et al*., 2005; Shetty *et al.,* 2000; Hemida, 1994; Abdel-Basset *et al.,* 1992). Diarak *et al.* (2001) indicated that insecticides Isofenfos and Phorate had no inhibitory effects on the development of soil microorganisms. Chen

[1] Agricultural and Natural Resources Research Center of Guilan, Rasht, P.O Box: 41635-3394, Islamic Republic of Iran.

[*] Corresponding author, e-mail: mhoushiarfard@yahoo.com

[2] Cotton Research Institute of Iran, Gorgan, Islamic Republic of Iran.

Jian *et al*. (1998) studied insecticidal effects of Iprobenfos and Tolcofos-methyl fungicides on rice brown plant hopper. The simultaneous effects of Dimethoate and Benomyl on soil organisms showed a reduction in collembola populations (Martikainen *et al*., 1998). The insecticide Selecron (Profenfos) caused a significant reduction in the total-N of *Penicillium chrysogenum* (Abdel-Malek *et al.,* 1994). The application of the recommended concentrations of Dimethoate decreased the percentage of soybean colonization by the vesicular arboscular mycorrhizal fungi (Menendez *et al.,* 1999). Khallil (1993) found that there was no appreciable effect of a low dose of Dimethoate (2.5 ppm) on vegetative growth, asexual and sexual sporulation of zoosporic fungi. Hexachlorocyclohexane, Carbofuran and Fenvalerate stimulated populations of *Penicillium* spp. in soil. The use of Fenvalerate inhibited the growth of *Fusarium* spp. but all insecticides reduced the population of *Rhizopus* in soil (Das and Mukherjee, 1998). The purposes of this study were to determine whether insecticides Thiodicarb and Imidacloprid had any possible effects on the most important fungi involved in diseases of cotton seedlings in Iran as well as their efficacy in reducing seedling death, resulting in increase in seedling emergence percentages.

MATERIALS AND METHODS

In Vitro Studies

Three soil fungi namely: *Rhizoctonia solani* Kuhn, *Fusarium moniliforme* and *Pythium ultimum* Trow, were isolated from cotton seedlings (*Gossypium hirsutum* L.) that showed the typical damping-off symptoms and chosen as the test fungi on account of their high frequency. To identify the pathogenic fungi in cotton seedlings, 47 infected seedlings were collected. Samples of stem or root were cut into 3-5 mm pieces and surface-sterilized in 0.5-1 % (w/v) aqueous NaOCl for 1-3 minutes, then rinsed in S.D.W and damp dried on filter paper followed by some of them being planted on W.A. 2 %, acidic PDA or CMA-PARP culture media and incubated at 23±1°C for 4-7 days. The isolates were purified and identified based on the morphological characteristics of sexual and asexual organs, cardinal temperatures for growth and colony morphology according to the classification scheme of monographs. Inoculation tests were carried out under greenhouse conditions to determine the pathogenicity of isolates using proper inoculation methods. The frequency of isolates of *R. solani*, *F. moniliforme* and *P. ultimum* were 60.1, 11.3 and 20.4 %, respectively. The two insecticides of Thiodicarb (Larvin® DF 80) and Imidachlopride (Guacho® SW 70) of 4 concentrations of 0, 5, 6 and 7/1,000 (gr. commercial product 1,000 ml^{-1} solution) equal to 0, 4,000, 4,800 and 5,600 mg a.i 1,000 ml^{-1} and 0, 3,500, 4,200 and 4,900 mg a.i 1,000 ml^{-1} (recommended rates of early season seed treatment for thrips) were used in the experiments, respectively (concentration of water never exceeded 0.2% (v/v) in the medium). The culture medium used in the study was Czapeck-Dox Agar (CDA) while de-ionised water being used as solvent for the insecticides. After the medium had cooled to 50°C and while still liquid it was treated with the desired volumes of 3 different concentrations from stock solutions of insecticides prepared in sterile distilled water. About 20 ml of each insecticide-agar mixtures were poured into each of five the 9-cm glass Petri dishes, to give replicates. Plates were inoculated by transferring five-millimeter diameter discs (containing hyphal tips of the test fungal species from less than 1-week-old cultures) on PDA plates. The plates were incubated in dark at 23±1°C. At the end of incubation period, depending on the growth rate of each species on the control plate (untreated with insecticides), the diameter of each fungus colony was measured, and the inhibition of mycelial growth (against control) was

[DOR: 20.1001.1.16807073.2011.13.1.1.4]

calculated by the equation: (C–T/C)×100 where C is control growth rate (mm) and T the treated growth rate (mm) of insecticides in the treated plates (Vincent, 1947). The mean percent inhibition values were initially changed into $\mathrm{ArcSin}\sqrt{\mathrm{Percentage}}$, then variance analysis (ANOVA) was carried out by using MSTAT-C software package and means compared by Duncan's Multiple Range Test.

Pot Culture Experiment

This study was carried out to determine the impacts of Thiodicarb and Imidacloprid on pre- and post-emergence damping-off between germination and emergence stages and as well after emergence of the cotton seedlings, respectively. The certified cotton seeds of *cv.* Sahel (*G. hirsutum* L.) were treated with two levels of the formulated preparations of each insecticide (in terms of equivalent dosages of their commercial product per kg of seed), selected as based on the figures 1 and 2 obtained from *in vitro* evaluations namely: Thiodicarb 4.8 and 5.6 and Imidacloprid 4.2 and 4.9 g a.i kg^{-1} seed. For each treatment, 50 g of seed was shaken vigorously with 15 ml of aqueous suspension containing the required amount of an insecticide in glass bottle until the insecticide suspension was completely adsorbed by the seeds. Inoculum of each pathogen was grown separately on autoclaved sand (6 g)-maize meal (4o g+75 ml water) in 250-ml flasks (1 hour on three successive days). CFU or PFU were determined by suspending 0.1 g of colonized cornmeal-sand in 9.9 ml of S.D.W and plating dilutions onto PDA or selective media. The sterilized sandy loam soil was thoroughly mixed with 5, 1 and 6% (w/v) of inoculum of *F. moniliforme*, *P. ultimum* and *R. solani*, to obtain the desired inoculum (Koyeas and Davatzi-Helena, 1980). Five treated seeds were sown in each 20 cm pot containing artificially infected soil in the greenhouse and regularly watered. The pots were arranged as a randomized design of 3 replicates for each insecticide treatment and for the untreated control. The number of seedlings that emerged in each pot was recorded in 7 and 15 days after sowing. The seedling emergence and relative efficacy of insecticides against the phenomenon of either pre- or post-emergence damping-off were assessed by the following equations:

Emergence (%)= 100× (Number of emerged seedling/Total number of sown seeds) (1

100- Emergence (%)= Pre-emergence damping-off % (2

Post- emergence damping-off (%)= 100×[(A-B)/A] (3

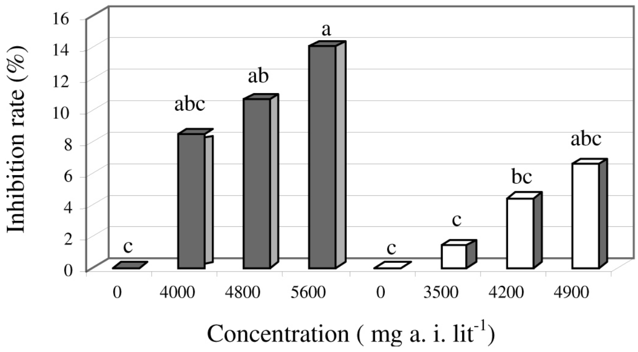

Figure 1. Effect of insecticide type×insecticide concentration interaction on the growth inhibition rate (%) of *Rhizoctonia solani* on CDA medium.
■: Thidicarb; □: Imidacloprid; * Mean of 5 replicates; Similar letters are not significantly different at 5% probability level.

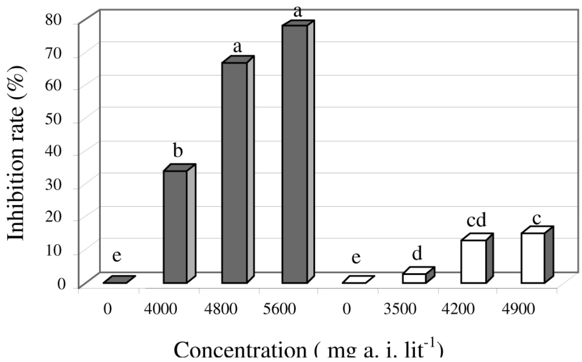

Figure 2. Effect of insecticide type×insecticide concentration interaction on growth inhibition rate (%) of *F. moniliforme* on CDA medium.
■: Thidicarb; □: Imidacloprid; * Mean of 5 replicates; Similar letters are not significantly different at 5% probability level.

Where *A* and *B* are the number of healthy and diseased seedlings, respectively.

Disease control (%) = 100×[(X-Y)/X] (4

Where *X* and *Y* are % damping-off in infested pots sown with the insecticide-treated and untreated seeds, respectively.

RESULTS AND DISCUSSION

There were significant differences of inhibition rate between the two insecticides for their effects on mycelial growth of *R. solani* and *F. moniliforme* (Table 1). There were also significant differences observed among the species in susceptibility to different concentrations of insecticides (Table 1). The percentage of mycelial growth inhibition varied among fungal isolates and insecticides (Tables 1 and 2). In other words, the insecticides inhibited (to some extent) the growth of most fungal species, although various degrees of growth inhibition could be noticed. Based on the number of species inhibited, Thiodicarb was found to be more effective than Imidacloprid and inhibited the growth of *F. moniliforme* as well as *R. solani* in the ranges of 34.1-78% and 8.5-14.1%, respectively (Table 2). *F. moniliforme* was more affected by the insecticides, while no significant effect of insecticides used on *P. ultimum* was

Table 1. Mean comparison of the effect of insecticide type on the growth of fungal pathogens on CDA medium.

Insecticide	*R. solani*	*F. moniliforme*	*P. ultimum*
		Growth inhibition rate (%) [a]	
Thiodicarb	11.1 a	59.7 a	0 a
Imidacloprid	4.1 b	10.3 b	0 a
Concentration of insecticide (g commercial product lit^{-1}) [b]			
0	0 c	0 c	0 a
5	4.9 b	18.5 b	0 a
6	7.6 ab	39.9 a	0 a
7	10.4 a	46.6 a	0 a

[a] Mean of 5 replicates.
[b] Concentration of insecticide in CDA (g Commercial product lit^{-1}); Thiodicarb:0, 4,000, 4,800 and 5,600 mg a.i lit^{-1} and Imidacloprid: 0, 3,500, 4,200, 4,900 mg a.i lit^{-1}.
Means followed by similar letters in each column are not significantly different at 5% probability level.

Table 2. Efectiveness of insecticidal seed treatments on pre- and post-emergence damping-off of cotton cv. Sahel (*Gossypium hirsutum* L.) in greenhouse conditions

Treatment	Dosage (mg a.i kg^{-1}seed) [a]	Seedling emergence % [b]	Pre-emergence damping-off [b]	Post-emergence damping-off [b]
			Disease Control %	
thiodicarb	4800	19.6 b	29.4 b	41.3 a
	5600	36.4 a	40.9 a	40.6 a
imidacloprid	4200	17.5 b	4.2 c	5 c
	4900	23.2 b	10.6 c	15 b
Check	0	16.4 b	-	-

[a] Mean of 3 replicates.
[b] 7-day and 15-day: Data recorded 7 and 15 days after sowing.
Similar letters in each column are not significantly different at 1% probability level

observed. The two high concentrations of each insecticide exerted the most inhibition rates (%) on fungi (Table 1). *R. solani* and *F. moniliforme* were srongly inhibited by the insecticides so that, the Thiodicarb showed the strongest activity against *F. moniliforme* (6 times higher than that of Imidacloprid) (59.7%; Figure 3).

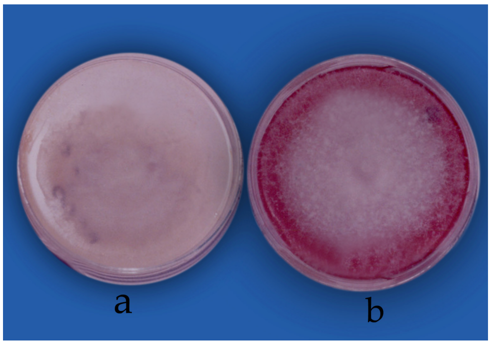

Figure 3. Effects of high concentrations of Thiodicarb (a) and Imidacloprid (b) on mycelial growth of *F. moniliforme* on CDA medium.

Efficacy of Insecticides against Damping-off Disease

With introduction of insecticides into the seed and plant environment a few possible interactions are conceivable. The results of pot experiments in the greenhouse conditions revealed that survival of seedlings in pot treated seeds with insecticides and fungal pathogens was significantly (P= 0.05) greater than the untreated ones. Thiodicarb provided excellent protection against pre- and post-emergence damping-off and seedling root rot by 54.2% and 90.6 %, respectively (Table 2). The treatments control and Imidacloprid had the least emergence and the rate of seed rot and pre-emergence damping-off in 15 days after sowing. As shown by the *in vitro* results, the two insecticides were completely ineffective against *Pythium* pre-emergence seed and seedling root rot. The *in vivo* performance of the two insecticides as seed treatment against damping-off and seedling root rot corresponded to some degree with their *in vitro* fungistatic activity against mycelial growth of *R. solani* and *F. moniliforme* isolates. The study confirmed the higher effectiveness of Thiodicarb as compared to Imidacloprid in reducing post-emergence damping-off of cotton seedlings. Thiodicarb exhibited excellent *in vivo* efficacy against pre-emergence damping-off disease. Thus, in the case of Thiodicarb, a strong *in vitro* effect can directly explain the *in vivo* potency for a considerable degree of damping-off disease control. It is apparent that pathogen-insecticide interaction can exist under *in vitro* and greenhouse conditions. However, because of adsorption

[DOR: 20.1001.1.16807073.2011.13.1.1.4]

and metabolism phenomena in soils, these insecticides exhibit different effects in soil. The differences between results of *in vitro* and pot experiments are due to the difference between physiology of saprophytic growing of fungal hyphae in culture medium and infectious papagules for parasitizing host tissue in *in vivo* conditions.

REFERENCES

1. Abdel-basset, R., Moharram, A. M. and Omar, S. A. 1992. Effect of Selecron on Cellulose Production and Respiration of Three Mesophilic Fungi. *Bull. Fac. Sci. Assiut. Univ.,* **21:** 145-153.
2. Chen Jian, M., Yu, X., Lu Zhong, X. and Zheng Xu, S. 1998. Insecticidal Effect of Paddy Field Fungicides on Nymph of Rice Brown Plant Hopper. *Chin. J. Rice Sci.,* **12:** 155-158.
3. Colyer, P. D. 1988. Frequency and Pathogenicity of *Fusarium* spp. Associated with Seedling Disease of Cotton in Louisiana. *Plant Dis.,* **72:** 400- 402.
4. Das, A. C. and Mukherjee, D. 1998. Insecticidal Effects on Soil Microorganisms and Their Biochemical Process Related to Soil Fertility. *World J. Microbiol. Biotech*., **14:** 903-909.
5. Diarak, M. and Kazanici, F. 2001. Effect of Some Organophosphorus Insecticides on Soil Microorganisms. *Turk. J. Biol.* **2:** 51-58.
6. Hawkins, B. S., Peacock , H. A. and Steele, T. E. 1966. Thrips Injury to Upland Cotton (*Gossypium hirsutum* L .) Varieties. *Crop Sci.,* **6:** 256-258.
7. Hector, R. F., Davidson, P. and Johnson, S. M. 2005. Comparison of Susceptibility of Fungal Isolates to Lufenuron and Nikkomycin Z Alone or in Combination with Itraconazol. *Am. J. Vet. Res.,* **66:** 1090-1093.
8. Hemida, S. K. 1994. Influence of Pyrethroid Insecticides on Soil Fungi. *Water Air and Soil Pollution*, **76:** 3-4.
9. Johnson, L. F., Maird, D. D., Chambers, A. V. and Shamiyeh, N. B. 1970. Fungi Associated with Post-emergence Seedling Disease Caused by *Pythium ultimum. Phytopathol.,* **69:** 298-300.
10. Khallil, A. M. A. 1993. Influence of the Insecticide Dimethoate on Some Metabolic Activities of Five Zoosporic Fungi. *J. Islamic Academy Sci.*, **5:** 256-269.
11. Koyeas, J. H. and Davatzi-Helena, P. 1980. Evaluation of Fungicides for Cotton Seed Treatments against Aoil-borne Fungi. *Annals Institute Phytopathologique Benaki,* **12:** 169-178.
12. Martikainen, E., Haimi, J. and Ahtianen, J. 1996. Effects of Dimethoate and Benomyl on Soil Organisms and Soil Process. *Proc. XII Int. Coloquim on Soil Zoology*, 22-26 July 1996 Dublin. *Appl. Soil Ecol.***, 9:** 381-387.
13. Menendez, A., Martinez, A., Chiocchio, V., Venedikian, N., Ocampo, J. A. and Godeas, A. 1999. Influence of the Insecticide Dimethoate on Arbuscular Mycorrhiza Colonization and Growth of Soybean Plants. *International Microbiol.,* **2:** 43-45.
14. Moharram, A. M., Abdel-malek, A. Y., Abdel-Kader, M. I. A. and Omar, S. O. 1994. Effect of Three Insecticides on Nitrogen Content of Some Soil Fungi. *J. Islamic Academy Sci*., **7:** 82-87.
15. Rummel, D. R. and Quisenberry, J. 1979. Influence of Thrips Injury on Leaf Development and Yield of Various Cotton Genotypes. *J. Econ. Entomol.,* **72:** 706-709.
16. Shetty, P. K., Mitra, J., Murthy, N. B. K., Namitha, K. K., Savitha, K. N. and Rugha, K. 2000. Biodegradation of Cyclodiene Insecticide Endosulfan by *Mucor thermohyalospora* MTCC 1384. *Current Sci.,* **79:** 1381-1382.
17. Vincent, J. H. 1947. Distortion of Fungal Hyphae in the Presence of Certain Inhibitors. *Nature,* **15:** 850.

اثرات دو حشره کش سیستمیک بر بیمارگرهای قارچی گیاهچه پنبه

م. هوشیار فرد و ت. درویش مجنی

چکیده

در این تحقیق اثرات متقابل بین دو حشره‌کش سیستمیک تیودیکارب (Larvin DF 80) و ایمیداکلوپراید (Gaucho SW 70) با قارچهای *Rhizoctonia solani*، *Fusarium monoliforme* و *Pythium ultimum* عوامل مرگ بیماری گیاهچه پنبه، در شرایط درون شیشه ای و گلخانه ای مورد بررسی قرار گرفت. نتایج نشان داد که این دو حشره کش به ترتیب به میزان ۱۴/۱–۶/۶ و ۱۵/۲–۷۰/۸ از رشد میسیلیومی گونه‌های *R. solani* و *F. moniliforme* جلوگیری کردند. حشره‌کش‌ها اثر بازدارندگی بیشتری روی رشد *F. moniliforme* داشتند در حالی که اثر معنی داری روی *P. ultimum* مشاهده نشد. آزمایشات گلدانی نتایج آزمایشات درون شیشه ای را تایید کرد به طوری که حشره کش تیودیکارب به ترتیب به میزان ۵۴/۲ و ۹۰/۶ درصد باعث کاهش بیماری مرگ گیاهچه در قبل و بعد از خروج از خاک شد و حفاظت مطلوب تری را فراهم کرد.

[DOR: 20.1001.1.16807073.2011.13.1.1.4]