$\Phi YTON$

REVISTA INTERNACIONAL DE BOTÁNICA EXPERIMENTAL INTERNATIONAL JOURNAL OF EXPERIMENTAL BOTANY

FUNDACION ROMULO RAGGIO Gaspar Campos 861, 1638 Vicente López (BA), Argentina www.revistaphyton.fund-romuloraggio.org.ar

Some physiological effects of acetamiprid on two cultivars of corn plants

Algunos efectos fisiológicos de acetamiprid en dos cultivares de plantas de maíz

Turkyilmaz Unal B¹ & A Esiz Dereboylu²

Abstract. The aim of the study was to investigate some physiological effects of an insecticide (Akira 20-SP) with acetamiprid agent on Cin misir Adapop 10 and Sert misir Adapop 2 cultivars of corn plants in different concentrations [0 g/L, 0.6 g/L (recommended concentration), 1.2 g/L]. Photosynthetic pigment substances, total soluble protein and proline contents were determined. Analysis of treated leaves showed that the recommended concentration resulted in higher chlorophyll value in Cin misir Adapop 10 and Sert misir Adapop 2. Carotenoid amounts increased in Cin misir Adapop 10 and decreased in Sert misir Adapop 2 to the control. Total protein contents decreased in x2 recommended concentration in Cin misir Adapop 10 and in all treatment groups in Sert misir Adapop 2 and proline amounts significantly increased in proportion to the increasing concentration in both cultivars. Especially high concentrations of acetamiprid affect plant metabolism of both cultivars negatively.

Keywords: Insecticide; Photosynthetic pigment; Proline; Protein; $Zea\ mays\ L.$

Resumen. El objetivo de este estudio fue investigar algunos efectos fisiológicos de un insecticida (Akira 20-SP) con acetamiprida a diferentes concentraciones [0 g/L; 0,6 g/L (concentración recomendada), 1,2 g/L] en dos cultivares de maíz (Cin misir Adapop 10 y Sert misir Adapop 2). Se determinaron pigmentos fotosintéticos, proteína total soluble y contenido de prolina. Los contenidos de clorofila total y de carotenoides fueron mayores en las hojas expuestas a las concentraciones recomendadas para Cin misir que en sus controles. Contrariamente, el doble de la concentración recomendada para Sert misir mostró menores valores para clorofila total y carotenoides que sus controles. Los contenidos de proteína total disminuyeron en el doble de la concentración recomendada de Cin Misir Adapop 10 y en todos los tratamientos en Sert misir Adapop 2. El contenido de prolina incrementó significativamente en plantas de ambos cultivares tratados con acetamiprida en comparación al control. Este estudio mostró que concentraciones altas de acetamiprida tuvieron algunos efectos negativos en la fisiología de plantas de maíz. El uso de pesticidas debería ser controlado siguiendo los estándares de países desarrollados para proteger la salud y el ambiente.

Palabras clave: Insecticida; Pigmento fotosintético; Prolina; Proteína; Zea mays L.

¹Nigde University, Ulukısla Vocational School, Plant and Animal Production Department, Nigde, Turkey.

² Ege University, Science Faculty, Biology Department, Bornova-Izmir, Turkey.

INTRODUCTION

Modern agriculture largely relies on the extensive application of agrochemicals, including inorganic fertilizers and pesticides. Insecticides are a pesticide used against insects. The use of insecticides is believed to be one of the major factors behind the increase in agricultural productivity in the 20th century (van Emden & Pealall, 1996). The widespread use of insecticides over the past 30 years has resulted in problems caused by their interaction with natural biological systems. They can harm plants and animals ranging from beneficial soil microorganisms and insects, nontarget plants, fish, birds, and other wildlife. Also, pesticides are one of the reason for water pollution and soil contamination (Ware & Whitacre, 2004; Sanders, 2010).

Acetamiprid is a systemic and contact insecticide; its chemical formula C₁₀H₁₁ClN₄ belongs to the neonicotinoids group. Owing to its broad insecticidal spectrum and relatively low acute and chronic mammalian toxicity, acetamiprid is used widely in crop protection (Tomizawa et al., 2000; Fitzgerald, 2004; Yao et al., 2006). Though the half life of acetamiprid under field conditions was reported to be about 2.8-14 days (Singh & Kumar, 2008), the risk of its ambient pollution, principally in water, is still present (Seccia et al., 2005). Acetamiprid exposure has been proven to have an adverse effect on greenhouse workers spraying this insecticide (Marin et al., 2004), soil microorganisms (Yao et al., 2006) and beneficial insects (Fitzgerald, 2004). The adverse impact of insecticides on natural enemies can be mitigated through choice of the insecticide and the dosage, and timing of the insecticide application (Obrycki & Kring, 1998). Biological control and selective insecticides have proven to be compatible strategies in integrated pest management (IPM) programs (Giles & Obrycki, 1997).

However, reports in the literature on the effects of insecticides on plant growth are limited, although there have been some reports of increased height and flowering (VanTol & Lentz, 1999). Most reports simply state a tolerance of the chemical by the plant (i.e. non-mortality post-treatment) or improved seed vigor without further definition (Ayyappath et al., 2000; Revellin et al., 2001; Delgarde & Rouland-Lefevre,

2002; Foster et al., 2003). Although the effects of insecticides have been tested on non-target organisms, their effects on plant physiology and anatomy have not been tested before approval.

This study aimed to determine the effects of different concentrations of the acetamiprid insecticide on photosynthetic pigments, and total soluble protein and proline contents on corn plants.

MATERIALS AND METHODS

The study was conducted in the greenhouse of the Biology department, Ege University, Izmir, Turkey. Akira 20-SP, an insecticide with the acetamiprid agent which protects corn against aphits and other agents, was applied to two different cultivars of corn ('Cin misir Adapop 10' and 'Sert misir Adapop 2') at different concentrations [0 g/L, 0.6 g/L (recommended concentration), 1.2 g/L]. Corn plants (Zea mays L.) were grown from seeds (from Adapazari Corn Research Institution). Seeds were sterilized before sowing by first immersing them in 2% sodium hypochlorite for 5 min, and then making a repeated rinsing with distilled water. One hundred plants were maintained in a plot using a completely Randomized Block Design (RBD). The first application was carried out on one-month-old seedlings using a hand sprayer until all leaves became drop-wet. Three more applications were done with 10-day intervals using the same method. Plants were periodically observed and no pest infestation or disease infection were detected even on untreated plants. Photosynthetic pigments (Lichtenthaler, 1987), total soluble protein (Bradford, 1976) and proline amounts (Bates et al., 1973) on corn leaves were determined. There were four replications for all experiments. The data were analyzed with Tukey's Multiple Range Test (Tukey, 1954).

RESULTS AND DISCUSSION

Chlorophyll amounts on corn leaves treated with different concentrations of acetamiprid are presented in Table 1.

Table 1. Effects of different concentrations of Acetamiprid on corn leaf chlorophyll and carotenoid contents. Standard errors (±) are indicated.

Tabla 1. Efecto de diferentes concentraciones de Acetamiprida en el contenido foliar de clorofila y carotenoide. Los valores son promedios ± error estándar.

Groups	Chlorophyll a (mg/mL)	Chlorophyll b (mg/mL)	Total chlorophyll (mg/mL)	Carotenoid (mg/mL)
Cin misir Control	2.455 ± 0.383	0.916 ± 0.171	3.370 ± 0.554	10.779 ± 2.474
Cin misir Rec. Con.	3.113 ± 0.266*	1.144 ± 0.038	4.257 ± 0.304*	14.134 ± 1.014*
Cin misir Rec. Con.x2	2.608 ± 0.126	0.893 ± 0.299	3.500 ± 0.155	11.386 ± 0.701
Sert misir Control	3.765 ± 0.117	1.240 ± 0.025	5.004 ± 0.143	14.467 ± 0.578
Sert misir Rec. Con.	3.414 ± 0.171	1.746 ± 0.821	5.159 ± 0.992	12.231 ± 2.734
Sert misir Rec. Con.x2	2.446 ± 0.272*	0.842 ± 0.019	3.287 ± 0.292*	10.574 ± 0.576*

^{*} Significantly different from control at alpha 0.05 according to Tukey test.

^{*} Significativamente diferente al control (α= 0,05 de acuerdo al test de Tukey).

Total chlorophyll values were greater on leaves exposed to the recommended concentration than in its control in Cin misir and Sert misir (Table 1). Contrarily, the x2 recommended concentration showed lower total chlorophyll contents than its control in Sert misir (Table 1).

Different concentrations (5, 10 and 25 ppm) of aldicarb, carbofuran, phorate fensulfothion and fenamiphos were observed for their pesticidal effects on chickpea plants in terms of various plant growth parameters such as plant length and weight, pod numbers, root-nodulations and chlorophyll content (Tiyagi et al., 2004). Significant improvement in chlorophyll content and plant growth were noted at the lower concentrations (e.g., 5 and 10 ppm) of different pesticides, and 5 ppm concentration proved highly effective and non-phytotoxic. The phytotoxic effect was noted in those plants treated with 25 ppm concentration of all pesticides (Tiyagi et al., 2004). However, Schulz et al. (2007) reported that the increase in leaf chlorophyll was not significant in soybean which seeds had been treated with imidacloprid and thiamethoxam.

The lowest total chlorophyll amounts were found in the x2 recommended concentration in Sert misir (Table 1). Similarly, in a study of Gerbera plants applied with Triact 70, plant growth, flower formation and photosynthesis rate were reduced in x4 the insecticide application. This effect was probably due to decreased net photosynthetic rate and stomatal conductance (Spiders et al., 2008).

Carotenoid amounts increased in Cin misir Adapop 10 and decreased in Sert misir Adapop 2 in comparison to the control after insecticide application (Table 1). Tort and Turkyilmaz (2003) showed that carotenoid amounts increased in the recommended concentration of Captan fungicide on pepper plants. Seedlings of rice, early watergrass (thiobencarb-resistant and thiobencarb-susceptible biotypes, R and S, respectively),

and late watergrass (thiobencarb-resistant and thiobencarb-susceptible biotypes, R and S, respectively) were hydroponically exposed to clomazone at concentrations ranging from 0.08 to 7.9 μ M. β -carotene concentrations (μ g/g fresh weight) were measured after a 7-day exposure period. Clomazone inhibited β -carotene synthesis via inhibition of the non-mevalonate isoprenoid synthetic pathway (TenBrook & Tjeerdema, 2005).

The results presented in Figure 1 demonstrated that protein amounts decreased under the recommended concentrations and x2 those concentrations in Sert misir in comparison to its control. Similarly, a decline was noticed in total proteins and RNA content of wheat shoots as a function of the applied pesticides (Abo-El-Seoud & Frost, 1998). Also, protein amounts of Cin misir Adapop 10 were similar under recommended concentrations (0.325 mg/g) to the control (0.304 mg/g). Concentration of 2.5 (recommended concentration) as well as 5 g/L Captan fungicide showed higher protein contents on pepper leaves than the higher concentration and the control (Tort & Turkyilmaz, 2003).

Proline amounts significantly increased in proportion to the increasing concentration in Cin misir Adapop 10 and Sert misir Adapop 2 in Figure 1. The highest amounts of proline were 41.242 µmol/g in Cin misir Adapop 10, and 26.150 µmol/g in Sert misir Adapop 2. Similarly, the amounts of proline in Cin misir Adapop 10 and Sert misir Adapop 2 increased in all application groups of a fungicide with a thiram agent (Tort et al., 2006). Forty-five-day-old plants of *Glycine max* (soybean) were exposed to several Deltamethrin (synthetic pyrethroid insecticide) concentrations (0.00%, 0.05%, 0.10%, 0.15% and 0.20%) through foliar spray under field conditions. In the treated plants, lipid peroxidation, proline content and total glutathione content increased, whereas the total ascorbate content decreased, as compared with the con-

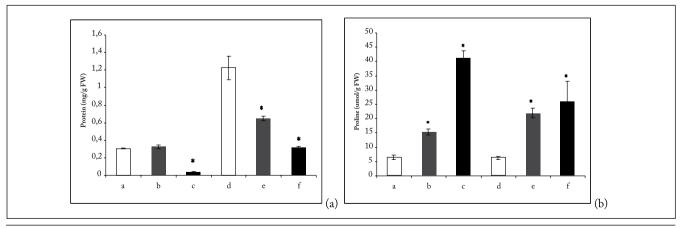


Fig. 1. Effects of different concentrations of Acetamiprid on corn leaf protein (a) and proline (b) contents. Standard errors (±) are indicated. * Significantly different from control at alpha 0.05 according to Tukey test. a: Cin misir Control, b: Cin misir Rec. Con., c: Cin misir Rec. Con.x2, d: Sert misir Control, e: Sert misir Rec. Con., f: Sert misir Rec. Con.x2.

Fig. 1. Efectos de diferentes concentraciones de Acetamiprida en el contenido foliar de proteína (a) y prolina (b). Los valores son promedios ± 1 error estándar. * Significativamente diferente al control (alfa = 0,05 de acuerdo al test de Tukey). a: Cin misir Control, b: Cin misir Rec. Con., c: Cin misir Rec. Con.x2, d: Sert misir Control, e: Sert misir Rec. Con., f: Sert misir Rec. Con.x2.

trols (Bashir et al., 2007). Also, analysis of leaves showed that the amounts of photosynthetic pigments and protein reduced, while the amount of proline increased under all concentrations of a fungicide with a Diniconazole agent on some barley cultivars (Tort et al., 2004).

In our study, treatment with insecticide concentrations yielded lower values of chlorophyll, carotenoid and protein amounts in the Sert misir cultivar of corn, except proline (Table 1 and Figure 1). Similarly, treatment with higher Captan fungicide concentrations yielded lower values of all investigated leaf contents in pepper (Tort & Turkyilmaz, 2003).

CONCLUSION

Human health and environment has gained great importance in the modern world. The widespread use of insecticides has resulted in problems caused by its interaction with the natural biological systems. Our study showed that higher amounts of acetamiprid have some negative effects on corn plant physiology. Thus, the use of pesticide should be controlled following the standards of developed countries to protect health and the environment.

REFERENCES

- Abo-El- Seoud & M. Frost (1998). Biochemical changes in wheat plants as affected by residues of dimethoate and pirimicarb. *Environmental Managment and Health* 9(5): 188-193.
- Ayyappath, R., S. Polavarapu & M. McGuire (2000). Effectiveness of thiamethoxam-coated spheres against blueberry maggot flies (Diptera: Tephritidae). *Journal of Economic Entomology* 93: 1473-1479.
- Bashir, F., Mahmooduzzafar, T.O. Siddiqi & M. Iqbal (2007). The antioxidative response system in *Glycine max* (L.) Merr. exposed to Deltamethrin, a synthetic pyrethroid insecticide. *Environmental Pollution* 147: 94-100.
- Bates, L.S., R.P. Waldern & I.D. Teare (1973). Rapid determination of free proline for water-stress studies. *Plant Soil* 39: 205-207.
- Bradford, M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Analytical Biochemistry* 72: 248-250.
- Delgarde, S. & C. Rouland-Lefevre (2002). Evaluation of the effects of thiamethoxam on three species of African termite (Isoptera: Termitidae) crop pests. *Journal of Economic Entomology* 95: 531-536.
- Fitzgerald, J. (2004). Laboratory bioassays and field evaluation of insecticides for the control of *Anthonomus rubi*, *Lygus rugulipennis* and *Chaetosiphon fragaefolii*, and effects on beneficial species, in UK strawberry production. *Crop Protection* 23: 801-809.
- Giles, K. & J. Obrycki (1997). Reduced insecticide rates and stripharvest: effects on arthropod predator abundance in Şrst-growth alfalfa. *Journal of the Kansas Entomological Society* 70: 160-168.
- Lichtenthaler, H.K. (1987). Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Method Enzymology* 148: 350-382.

- Marín, A., J.L.M. Vidal, F.J.E. Gonzalez, A.G. Frenich, C.R. Glass & M. Sykes (2004). Assessment of potential (inhalation and dermal) and actual exposure to acetamiprid by greenhouse applicators using liquid chromatography-tandem mass spectrometry. *Journal Chromatography b* 804: 269-275.
- Obrycki, J. & T.J. Kring. 1998. Predaceous Coccinellidae in biological control. *Annual Reivew of Entomology* 43: 295-321.
- Revellin, C., J.J. Giraud, N. Silva, P. Wadoux & G. Catroux (2001). Effect of some granular insecticides currently used for the treatment of maize crops (*Zea mays*) on the survival of inoculated *Azospirillum lipoferum*. *Pest Management Science* 57: 1057-1080.
- Sanders, H.O. (2010). Toxicity of pesticides to the crustacean *Gammarus lacustris*. Technical papers of the bureau of sport fisheries and wildlife, No. 25. US Dept. of Interior Fish and Wildlife Service, Washington D.C.
- Seccia, S., P. Fidente, D.A. Barbini & P. Morrica (2005). Multi residue determination of nicotinoid insecticide residues in drinking water by liquid chromatography with electrospray ionization mass spectrometry. *Analytica Chimica Acta* 553: 21-26.
- Singh, D.K. & S. Kumar (2008). Nitrate reductase, arginine deaminase, urease and dehydrogenase activities in natural soil (ridges with forest) and in cotton soil after acetamiprid treatments. *Chemosphere* 71: 412-418.
- Spiers, J.D., F.T. Davies, C. He, K.M. Heinz, C.E. Bogran & T.W. Starman (2008). Do insecticides affect plant growth and development? (Research tests foliar insecticides to determine whether applications affect development in Gerbera daisies). Greenhouse Grower, February, Vol:2.
- TenBrook, P.L. & R.S. Tjeerdema (2005). Comparative actions of clomazone on β-carotene levels and growth in rice (*Oryza sativa*) and watergrasses (*Echinochloa* spp). *Pest Management Science* 61(6): 567-571.
- Tiyagi, S.A., S. Ajaz & M.F. Azam (2004). Effect of some pesticides on plant growth, root nodulation and chlorophyll content of chickpea. Archives of Agronomy and Soil Science 50: 529-533.
- Tomizawa, M., D.L. Lee & J.E. Casida (2000). Neonicotinoid insecticides: molecular features conferring selectivity for insect versus mammalian nicotinic receptors. *Journal of Agriculture and Food Chemistry* 48: 6016-6024.
- Tort, N., A.E. Dereboylu & B. Türkyılmaz (2006). Morphological and physiological effects of a fungicide with a Thiram agent on some corn culture forms. Ege University Journal of Faculty of Science 29: 67-79.
- Tort, N. & B. Turkyilmaz (2003). Physiological effects of Captan fungicide on pepper (*Capsicum annuum* L.) plant. *Pakistan Journal of Biological Science* 6(24): 2026-2029.
- Tort, N., B. Türkyılmaz, A. Dereboylu & N. Tosun (2004). Morphological and physiological effects of a fungicide Diniconazole agent on some barley culture forms. *Ege University Faculty of Agriculture Electronic Journal* 41(1): 169-179.
- Van Emden, H.F. & D.B. Pealall (1996). Beyond Silent Spring. London: Chapman & Hall. 322 p.
- Van-Tol, N. B. & G.L. Lentz (1999). Evaluation of adage 5FSTMfor early season insect control. In: P. Dugger and D. Richter (eds.), Beltwide Cotton Conferences, pp. 1098-1100. Memphis, TN: National Cotton Council.
- Ware, G.W. & D.M. Whitacre (2004). The Pesticide Book, 6th ed. Willoughby, OH: Meister Media Worldwide. p. 496.
- Yao, X., H. Min, Z. Lü & H. Yuan (2006). Influence of acetamiprid on soil enzymatic activities and respiration. *European Journal of Soil Biology* 42: 120-126.