Comparative study of biological activity of fluorinated 5-*aminopyrazoles* on *Spodoptera frugiperda*

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Abstract— *Several derivatives aminopyrazoles, which are a new class of synthetic insecticide agents, have shown biological activity against lepidopteran, such as Spodoptera frugiperda. The aim of this work was to assess the biological activity of these synthesized compounds. For this purpose, derivatives of 5-amino-1-aryl-1H-pyrazole-4-carbonitriles were synthesized from commercially available aryl hydrazines and (ethoxymethylene)malononitrile. Three compounds (A, B, C) were tested for in vitro biological activity; two of them, A [5-amino-1-(2,6-dichloro-4-(trifluoromethyl)phenyl)-1H-pyrazole-4-carbonitrile] and B [5-amino-1-(perfluorophenyl)-1H-pyrazole-4-carbonitrile] showed promising activity, particularly the compound A.*

Keywords— biological activity, aminopyrazole, Spodoptera frugiperda, polyphagous lepidopteran.

I. INTRODUCTION

The new sowing methods have increased the incidence of at least two species of lepidopteran in corn crops: the fall armyworm, *Spodoptera frugiperda*, and the corn earworm, *Heliothis zea*. The fall armyworm (FAW) *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae) is a widespread plague that attacks maize (*Z. mays L.)*, which is grown in summer and winter crops (Campos, Ferreira, Costa, & Lasmar, 2014). The fall armyworm is a highly migratory polyphagous lepidopteran. It can colonize over 80 different plant species including grasses and crops such as alfalfa, soybean, sorghum, and corn. It is a harmful insect for sweet corn and vegetable crops (Bohnenblust & Tooker, 2012). It can be found in Southern USA, México, Central and South America (Murúa, Molina-ochoa, & Coviella, 2006), (Farías et al., 2008). The fall armyworm affects the quality of plants because the larvae feed on leaf margins and reduces the plant vigour. The greatest damage, though, is caused when they bite the crown of the plants, which causes weakness, poor development and, eventually, the death of the plant (Kumar & Mihm, 2002). Larval densities are often reduced to one or two per plant in heavy infestation as larvae can exhibit cannibalistic behaviour (Bohnenblust & Tooker, 2012).

It is well known that this lepidopteran is very useful for preliminary studies on new target compounds for insecticidal activity. Particularly, *Spodoptera frugiperda* has been used for many bioassays with *N*-phenylpyrazoles (Jensen-Korte et al., 1990) (Leslie et al., 1999)·(Wang et al., 2013). As part of our research project in sustainable agrochemistry in a previous work, a series of *5-amino-1-aryl-1H-pyrazole-4-carbonitrile* fluorinated were synthesized from aryl hydrazine and (ethoxymethylene)malononitrile (Plem, Müller, & Murguía, 2015) using a simple methodology. As excellent stereoselectivity and yields were obtained, we tested some of the compounds containing fluorinated atoms in the aryl part of molecule. These compounds are considered to be precursor analogues to Fipronil. Fipronil was the first phenylpyrazole introduced in the market for pest control (Jiang, Zheng, Shao, Ling, & Xu, 2014). It is noteworthy that substances belonging to the phenylpyrazole family have mainly herbicidal effects, whereas Fipronil is a powerful insecticide (Simon-Delso et al., 2015).

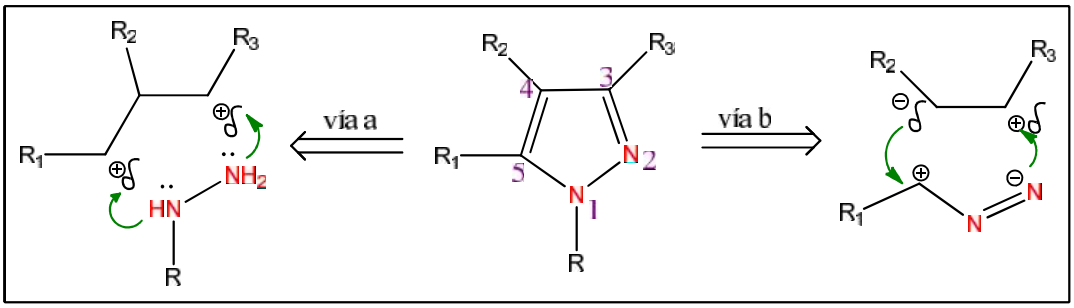
In this work, the *5-amino-1-aryl-1H-pyrazole-4-carbonitrile* synthesis was tested on the fall armyworm *Spodoptera frugiperda*.

II. MATERIALS AND METHODS

2.1 Chemical Synthesis

Among pyrazole derivatives, *N*-aryl-5-aminopyrazole fragment occupies a prominent position because it constitutes the core structure of a vast number of biologically active compounds(Marinozzi, Marcelli, & Carotti, 2015)'(Aggarwal, Kumar, Kumar, & Singh, 2011).

Pyrazole, a five-membered heterocycle with two adjacent nitrogen atoms (Scheme 1), is a model found in a number of small molecules that possesses a wide range of agricultural and pharmaceutical activities (Fustero et al., 2008)'(Okada, Okui, Takahashi, & Fukuchi, 1991)'(Fakhry Anwar & Hilmy Elnagdi, 2009). Conventional approaches for the synthesis of pyrazoles involve the bond C-N with condensation of hydrazines with 1,3-dicarbonyl compounds (Scheme 1, via a) or by intermolecular cicloadditions of 1,3-dipoles to dipolarophiles (Scheme 1, via b) (Yet, 2008)'(Elguero, Silva, & Tom, 2011).

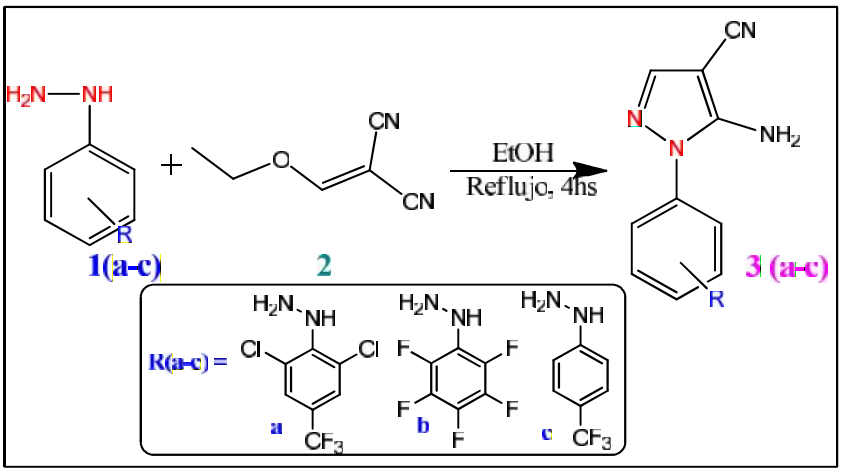


SCHEME 1. GENERAL PLATFORM FOR THE N-ARYLPYRAZOLE SYNTHESIS

2.1.1 General procedure for the synthesis of *5-amino-1-aryl-1H-pyrazole-4-carbonitrile*:

The aryl hydrazine (**1a-c**) [2,6-dichloro-4-(trifluoromethyl)phenyl]hydrazine.**1a**, (perfluorophenyl) hydrazine **1b**, and 4-(trifluoromethyl) phenylhydrazine **1c**]; i.e., (1.2 m mol) in absolute ethanol (2 mL) with stirring, and (ethoxymethylene) malononitrile (**2**) was added. Once the addition was complete, the solution was carefully brought to reflux, keeping nitrogen atmosphere.

The reaction mixture was refluxed for 4 hours. The reaction crude was purified by column chromatography on silica gel adsorption with a hexane/ethyl acetate gradient mixture as eluants. The following mentioned general procedure gave **3a-c** in 46, 65 and 80% yields, respectively (Scheme 2).



SCHEME 2. SYNTHESIS OF 5-*amino*-1-*aryl*-1 *H-pyrazole*-4-*carbonitrile* (3a-c).

2.1.2 General Procedures

All the products were characterized by ^{1}H NMR, ^{13}C NMR, ^{19}F NMR, COSY, HSQC, HMBC and MS spectral data. The yields were quantified by CG (internal standard method). GC analyses were performed on a DANI Master GC chromatograph equipped with 5 % diphenyl, 95 % dimethylpolysiloxane, a low bleed capillary column (30 m × 0.53 mm, 0.5

μm film thickness) and a flame ionization detector. Column chromatography was performed on silica gel (70 - 230 mesh ASTM), high-purity grade, pore size 60 Å. Ultra performance liquid chromatography and mass-spectrometry (UPLC-MS) analysis were performed on a H-CLASS SQD2 detector (Waters). ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz). ^{19}F NMR (300 MHz), COSY (Correlation spectroscopy), HSQC (heteronuclear single-quantum correlation spectroscopy) and HMBC (heteronuclear multiple-bond correlation spectroscopy) experiments were recorded at 20 °C on a Bruker Avance 300 MHz spectrometer in $CDCl_3$ using TMS as internal standard. Melting points (Mp) were recorded on a Büchi b-540 micro melting point apparatus and were uncorrected.

2.2 Bioassay

Tested insects: the larvae used for these tests were from a colony of *Spodoptera frugiperda*, bred for generations in the laboratory of the National Agricultural Technology Institute (INTA Castelar, Argentina). The population had not been previously exposed to insecticides. Laboratory FAW larvae were fed on corn flour and soya flour, wheat germ and yeast based artificial diet in a controlled room environment at 25 ± 3 °C, 60-70 % relative humidity, and 14/10 (L/D) of artificial photoperiod.

Evaluation of compounds: The compound **3a** [*5-amino-1-(2,6-difluoro-4-(trifluoromethyl)phenyl)-1H-pyrazole-4-carbonitrile*] was used for treatment A, as well as compound **3b** [*5-amino-1-(perfluorophenyl)-1H-pyrazole-4-carbonitrile*] was used in treatment B, and compound **3c** [*5-amino-1-(4-(trifluoromethyl)phenyl)-1H-pyrazole-4*-carbonitrile] for the treatment C.

For the bioassays, all the compounds (**3a**, **3b**, and **3c**) were dissolved in acetone of analytic grade at 20 ppm concentration. The insecticidal activity was tested against FAW, second instar larvae individually isolated. The topical application method was to apply 0.25 μL of each solution at 20 ppm on the pronotum of each larva. The compounds were applied with a Burkard hand operated micro-applicator of repetition.

They were tested by three repetitions on ten larvae, and there was also a treatment control larvae on which only acetone was applied. The larvae treated were kept individually isolated in plastic cups, fed with artificial diet and under the same environmental conditions within rearing chambers.

After 24 h of insecticide application, individual mortality was recorded. A dead larva was that one unable to move, displace or change position. The larvae were checked every day after treatment until finishing lepidopteran life cycle.

2.3 Statistical Data Analysis

To compare whether the observed differences in two or more survival curves can be explained as the result of a coincidence or as the effect of the evaluated compounds, the survival curves were compared using the log-rank test. The log rank test has the null hypothesis of no differences between populations for the occurrence of an event (i.e. survival) at any time during follow-up. The analysis is based on the timing of events (i.e. death) of each group are compared with the expected number of events if there were no differences between groups. The chi-square statistic is used to analyse the observed and expected deaths (Van Houwelingen, 1995)'(Lee & Wang, 2003).

Once the significant differences are accepted, the curve is modelled. The survival curve was remodelled using a Linear Regression Model. It incorporated a dummy variable that considers the treatment and control groups to be compared. The proposed model for observations:

$$Y = \alpha + \beta D + \gamma T + \delta DT + \varepsilon$$

where: D: number of days elapsed; T: treatment applied (T = 1 corresponds to the treatment applied for each compound, T = 0 corresponds to the control); Y: number of larvae that survive after D days, ε: random error term that is assumed to be normal for the two groups (control and treated) with variance-covariance matrix equal to $\sigma^2 I$ (Gujarati, 1970) (Suits, 1957).

The results were processed with the free software R, and LM (Linear Model) function; the predetermined level of statistical significance was 0.05 for all cases and the exact p-value was reported.

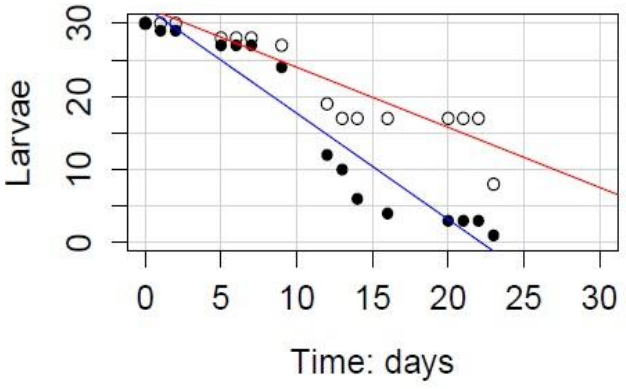
III. RESULTS AND DISCUSSION

When comparing the results of the groups of larvae treated with 20 ppm topical concentration with the control groups, using the log rank test, significant differences in the chi-square test and $p < 10^{-4}$ value were obtained. The survival curve was modelled by identifying the differences between control and treatment groups and using the model of linear regression. With treatment A the adjusted models for the two groups were:

$$Y \text{ (adjusted, control)} = 32.2 - 0.82D$$

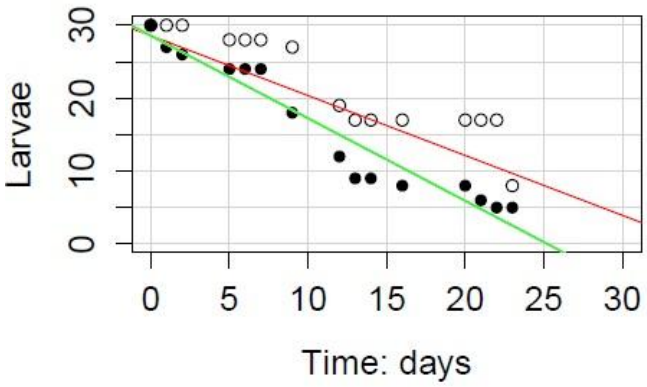
$$Y \text{ (adjusted, treatment A)} = 32.2 - 1.45D$$

The differences between the slopes were significant for p-value = 3.3×10^{-4} and the value of R-squared = 0.91; which indicates very good linear fit. Figure 1 shows the setting for data control and treatment for groups A.



o: Control; ●:Treatment A

FIGURE 1. SCATTER DIAGRAM WITH LINEAR FIT IN BOTH GROUPS: CONTROL AND TREATMENT A.



o: Control; ●:Treatment B

FIGURE 2. SCATTER DIAGRAM WITH LINEAR FIT FOR BOTH GROUPS: CONTROL AND TREATMENT B.

With the treatment B, the adjusted models for the two groups were:

$$Y \text{ (adjusted, control)} = 28.57 - 0.82D$$

$$Y \text{ (adjusted, treatment B)} = 28.57 - 1.13D$$

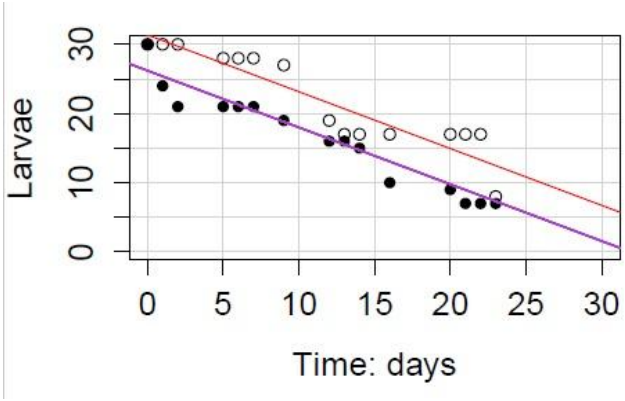
Differences between the slopes were significant, with p-value = 2.2×10^{-2} and the value of R-squared = 0.92 which indicates a very good linear fit. The Figure 2 shows the setting for the data of control and treatment groups B, together with the regression lines for each one.

For treatment C, the adjusted models for the two groups were:

$$Y \text{ (adjusted, control)} = 31.36 - 0.82D$$

$$Y \text{ (adjusted, treatment C)} = 26.20 - 0.82D$$

Differences between the slopes were not significant, the lines are parallel and the value of R-squared = 0.92; which indicates very good linear fit. Figure 3 shows the setting for the data of control and treatment groups C, together with the regression lines for each one.



o: Control; .:Treatment C.

FIG. 3: SCATTER DIAGRAM WITH LINEAR FIT FOR BOTH GROUPS: CONTROL AND TREATMENT GROUPS C.

IV. CONCLUSION

Several derivatives of 5-amino-*1-aryl-1H-pyrazole-4-carbonitriles* were synthesized from commercially available aryl hydrazines and (ethoxymethylene)malononitrile. Three compounds (A, B, C) were tested for *in vitro* biological activity; two of them, A [*5-amino-1-(2,6-dichloro-4-(trifluoromethyl)phenyl)-1H-pyrazole-4-carbonitrile]* and B [*5-amino-1-(perfluorophenyl)-1H-pyrazole-4-carbonitrile*] showed promising activity, particularly the compound A.

Based on the data analyzed, it can be seen that from the three compounds tested, A and B, were more active at 20 ppm topical concentration on *Spodoptera frugiperda*, because present more drastic effects on the population at tested concentration. Particularly, compound A is most active than B, if see the p-value ($3.3x10^{-4}$ of A, compared with $2.2x10^{-2}$ of B) the result was evident. So in the future can propose do the test with other concentration for evaluated more specifically.

The compound C showed no significant difference in slopes. Besides, it was observed an increase in the food consumed by the larvae, but no significant effects on lethality.

CONFLICT OF INTEREST

The authors claim that this article has no conflict of interest.

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