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CYCLOPENTANESPIRO-5-(2,4-DITHIOHYDANTOIN): SYNTHETIC PATHWAYS AND PHYTOTOXIC EXAMINATION TOWARDS OIL YIELDING ROSE (*ROSA DAMASCENA* MILL)

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Abstract

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The current paper presents a study on phytotoxic examination of synthetic compound cyclopentanespiro-5-(2,4-dithiohydantoin) (**cpsdth**) with regard to oil yielding rose. Different reaction pathways for **cpsdth** synthesis are given. Standard phytotoxicity vigor tests were conducted in order to determine any potential phytotoxic action of the compound.

Key words: cyclopentanespiro-5-(2,4-dithiohydantoin), *Rosa damascena*, phytotoxic examination

Abbreviations: cpsdth – cyclopentanespiro-5-(2,4-dithiohydantoin); HMDO - hexamethyldisiloxane;
LR – Lawesson's reagent

Introduction

Oil yielding rose (*Rosa damascena* Mill) is one of the most typical agricultural species in the Bulgarian region, and it is of significant economic importance. At present, registered plant protection products are deemed insufficient. What is more, research of possible phytotoxic action of novel pesticides is considered insufficient, as well.

In our previous research we have found out that the cyclopentanespiro-5-(2,4-dithiohydantoin) (**cpsdth**) shows a strong insecticidal activity against *Cladius pectinicornis*, a pest on roses of economic significance (Marinov et al., 2012). The **cpsdth** is a synthetic organic substance, belonging to the group of biologically active spirohydantoins and their thioanalogues. These are compounds with a proven application in the fields of human medicine and agriculture. Therefore, it is important to find out the optimal reaction conditions for obtaining the above mentioned compound in respect of its future application in agricultural practice. Considering this, our team developed three different synthetic pathways for obtaining **cpsdth** (Marinov et al., 2005; Marinov et al., 2012). These pathways are summarized and compared to each other in the current paper.

Experimental

Instrumentation and methods

All chemicals used were purchased from Merck and Sigma-Aldrich. The initial cyclopentanespiro-5-hydantoin was synthesized *via* the Bucherer-Lieb method (Bucherer and Lieb, 1934). Lawesson's reagent (LR) was prepared (Thomsen et al., 1990). The cyclopentanespiro-5-(2,4-dithiohydantoin) was synthesized using three different methods, presented in Figure 3 (see the results and discussion section).

Phytotoxic tests

As per the EPA Ecological Effects Test Guidelines: OPPTS 850.4150 Vegetative Vigor, Tier I (EPA OPPTS 850.4150, 1996)

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standard phytotoxicity vigor tests were conducted on oil-yielding rose plants (*Rosa damascena* Mill*)* in flowering growth stage. Ten samples of different plants were selected at random and were marked for test purposes. The shoots were sprayed using a standard laboratory sprayer with a delivery rate of 1000 l ha^{-1} to the point of runoff. Standard organosilicone surfactant Silwet®L-77 was added at 0.015 % concentration to the spray solutions to improve wetting ability. The control variant was sprayed only with distilled water with Silwet®L-77 at concentration 0.015 %.

Observations for any phytotoxic manifestations were conducted on every 7 days for a period of two consecutive applications (14 days). The length of shoots and shoots dry weight were determined at the end of the test. Ten different solutions (concentrations) of the product were prepared with distilled water, as follows: 0.001, 0.002, 0.003, 0.005, 0.007, 0.008, 0.020, 0.022, 0.024 and 0.025. The upper value is the most possible saturated concentration of the compound in water. Acute phytotoxic manifestations on plants were determined using the Percentage Disease Index formula (PDI) Mckinney infection index (Mckinney, 1923)

Mathematical manipulation of data

Data received from the conducted test were statistically manipulated with R language for statistical computing (R Development Core Team, 2011). Standard One-way ANOVA analysis was conducted in order to determine statistically proven diffuseness between the tested product concentrations and the control variant (Logan, 2010). Figure 1 depicts ANOVA analysis as conducted with R language Integrated Development Environment (R IDE) as per length shoots data, and Figure 2 – as per weight dry shoots data.

Results and Discussion

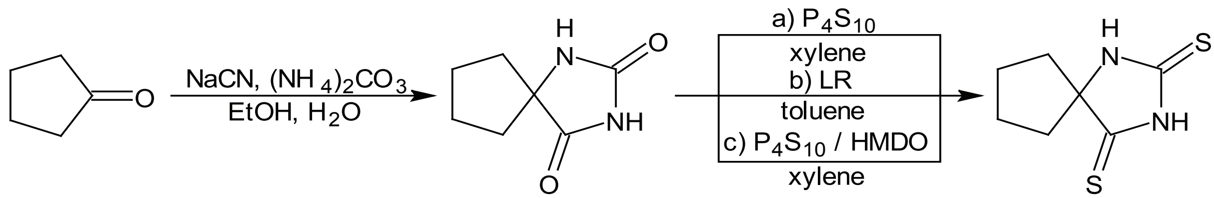
Synthetic techniques for obtaining cyclopentanespiro-5-(2,4-dithiohydantoin) (3), developed by us till now (Marinov et al., 2005; Marinov et al., 2012), are summarized in Figure 3. The first synthesis stage of **3** comprises the Bucherer-Lieb method (Bucherer and Lieb, 1934) as applied to cyclopentanone (**1**), which resulted in the formation of cyclopentanespiro-5-hydantoin (**2**). Thus, obtained product (**2**) was subjected to thionation *via* different reaction pathways. Method **a** is based on the interaction of **2** and P_4S_{10} (mole ratio 1:1) in xylene (Marinov et al., 2005). In method **b**, 2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane-2,4-disulfide (Lawesson's reagent, LR, Figure 4) was used as thionation reagent of **2** (mole ratio 1:2) **(**Marinov et al., 2005). In this case, the reaction was carried out in a medium of toluene. Method **c** is based on the treatment of **2** with the reagent combination of P_4S_{10} and hexamethyldisiloxane (HMDO) in xylene (Marinov et al., 2012**)**. The reaction conditions of these three methods are presented in Table 1.

```
> summary(aov(values~ind, data=cpsdth_length_stack))
            Df Sum Sq Mean Sq F value Pr(>F)
ind         10   11.8 1.18000  1.4281 0.1791
Residuals   99   81.8 0.82626
```

Fig. 1. ANOVA analysis by R – data length shoots

```
> summary(aov(values~ind, data=cpsdth_weight_stack))
            Df  Sum Sq  Mean Sq F value Pr(>F)
ind         10 0.26314 0.026314  1.3394 0.2204
Residuals   99 1.94489 0.019645
```

Fig. 2. ANOVA analysis by R – data weight dry shoots



1 2 3

Fig. 3. Synthetic pathways for obtaining of cyclopentanespiro-5-(2,4-dithiohydantoin)

Method **c** is obviously the most convenient synthetic procedure for obtaining of **3,** making it suitable for application into the agricultural practice. Through its application, the target product was obtained with a very high yield and the reaction time was significantly reduced as compared to the other techniques cited above. The compound **3**, synthesized *via* the methods mentioned above was characterized by physicochemical parameters and IR and NMR spectral data and the results obtained were identical (Marinov et al., 2005; Marinov et al., 2012).

As evident from the R language source code above, p-values calculated from the ANOVA analysis conducted are 0.1791 – for length shoots, and 0.2204 – for dry weight shoots. This is to prove that there is no significant difference between tested variants, including control. The Tukey HSD analysis conducted via R language function Tukey HSD indicates that at 95% confidence level there are no significant differences within all possible combinations of the test variants, and the respective length and dry weight data of the shoots.

TukeyHSD graphic results are depicted in Figure 5 – for shoots length, and in Figure 6 – for dry weight.

No visual phytotoxic manifestations were observed on plants on the 7th and the 14th day following treatment.

Conclusion

The results show the absence of a phytotoxic effect on oil yielding rose of a compound with potential insecticidal activity against a pest of economic scale for this plant culture. This

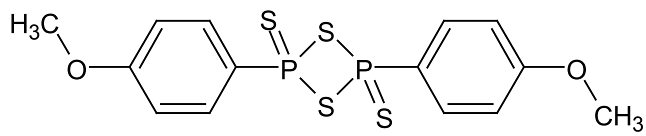


Fig. 4. LR

Table 1
Reaction conditions for thionation of 2

Synthetic procedure	Thionation reagent	Duration of the reaction	Medium	Solvent for recrystallization	Yield
Method a	P_4S_{10}	5 h	xylene		80 %
Method b	LR	6 h	toluene	methanol/water	88 %
Method c	P_4S_{10}/HMDO	1.5 h	xylene		92 %

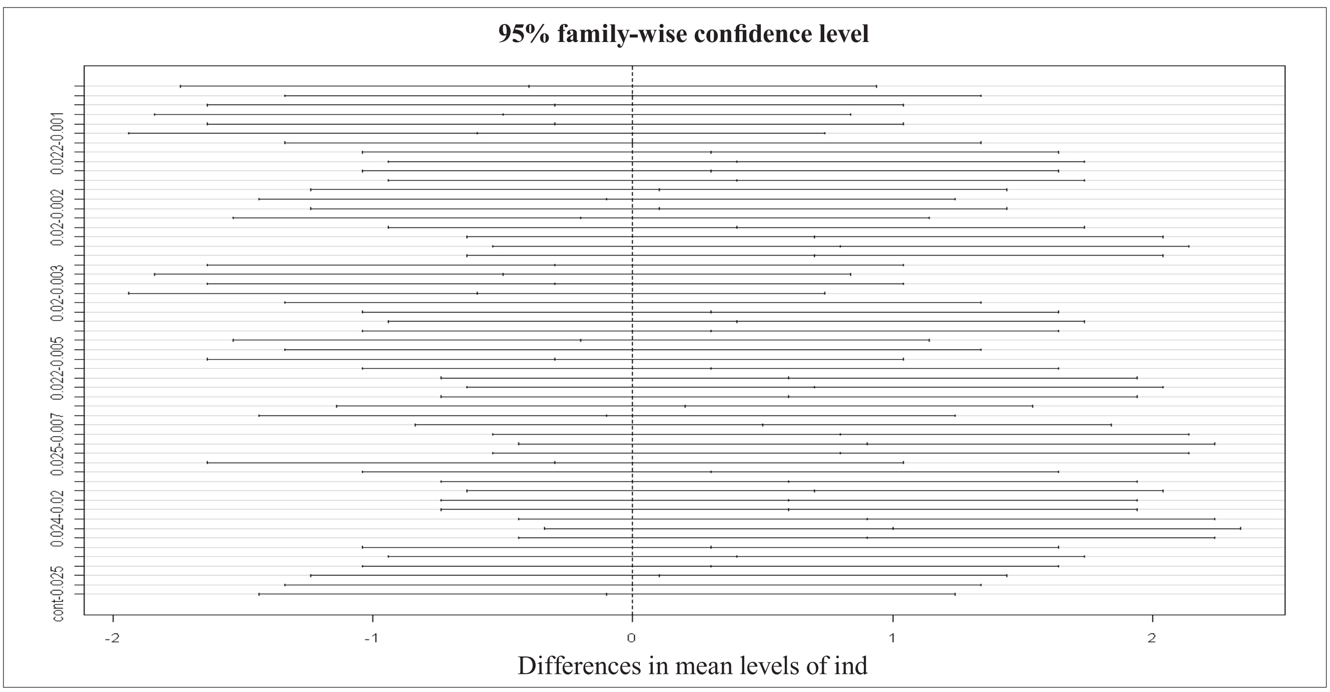


Fig. 5. TukeyHSD analysis - data length shoots

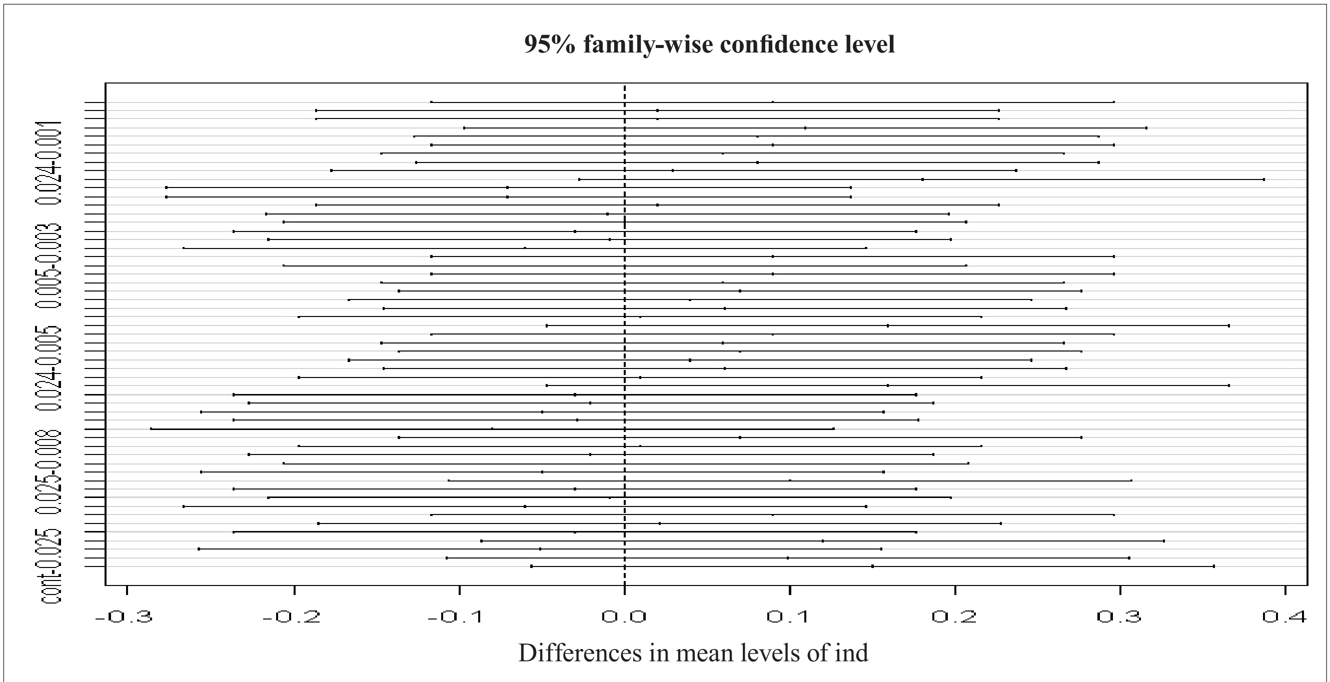


Fig. 6. TukeyHSD analysis - data weight dry shoots

clearly opens the way for future investigations in this area with the aim to create a novel plant protection product for oil yielding roses and their pest control.

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