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Article

Cymbopogon schoenanthus essential oil's effect on Bumble-bees; Preliminary results

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Abstract: Recently, it has become apparent that some pests especially insects can be controlled by exposure to small concentrations of the essential oils. What is unknown is whether these oils also affect pollinators. Our study on the effect of *Cymbopogon schoenanthus* (Poaceae) essential oil effect on the more adopted pollinator *Bombus terrestris* on greenhouse tomatoes crop in Biskra (arid region of Algeria) is part of an FNR project ran by the Center of Scientific and Technical Research on Arid Regions. The aim of this research is to assess *C. schoenanthus* (Poaceae) essential oil toxicity on bumblebees at concentrations of 0 µl/ml, 20µl/ml and 1000 µl/ml under laboratory conditions. Results showed that concentrations of 20µl/ml were not harmful while pure E O of *C. Schoenanthus*is was detrimental. The experimentation suggested here presents further evidence partisan the use of conditioning techniques to assess the use of essential oils on tomatoes greenhouse pests.

Keywords: *Cymbopogon schoenanthus, effect, Bombus terrestris*.

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1. Introduction

Bumblebees, like bees, appreciated by market gardeners. A very effective example is the interest in using *Bombus terrestris* for greenhouse tomato production. Thus, until 1988, a vibrating stick, called an "electric bee" was used manually for tomato flowers pollination, (self-fertilization). However, since the number of perfectly pollinated flowers is low, this system is insufficient. The first Bumblebees hives were introduced in 1988, resulting a clear increase in yield, size and weight of fruits. This also allowed the sale of tomato clusters, which was not possible with hand pollination because to obtain these clusters, it was necessary to go at definite times of the day and pollinate all the flowers of the inflorescences correctly, which represents a very high cost in labour [1].

In Algeria, the importation of bumblebees for greenhouse tomato pollination has been established. However, the short lifespan of beehives is observed by farmers who have adopted this trend in Biskra region. What is frustrating, however, is the simultaneous use of phytosanitary products with these bio-pollinators.

Meanwhile [2] mention that certain pesticides used in Italy under greenhouses have great toxicity to bumblebees even at minute doses. Likewise, [3] stated a wide variety of effects (lethal and sub-lethal) subsequent exposure to the pesticide.

Many papers described the effectiveness different types of plants' essential oils as alternative to synthetic pesticides as possessing insecticidal activity. Then; [4] declared since plants' essential oils are rich in bioactive compounds, there is potential for other compounds to be used as contact or fumigant pesticides. Thus many works present efficiency of different species of plants 'essential oils on insects mortality amongst them some reported the efficiency of the *Cymbopogon* genera like [5]; [6]; [7]; [8]; [9] and from Algeria;

[10] who reported that *Cymbopogon schoenanthus* EOs from Algerian Sahara showed effective insecticidal activity on the cowpea seed beetle; mainly the samples' EO from Tamanrasset.

Within the framework of developing an integrated management strategy for *Tuta absoluta*, and reducing the use of synthetic chemical pesticides CRSTRA ran a project to verify insecticid effect of *C. schoenanthus* essential oil on this tomatoes hard pest. Preliminary results are promising (unpublished data). At the same time bumble bees toxicity by synthetic pesticides has been reported by many authors through different routs even with concentrations of pesticides that may be considered safe for bumblebees or lesser than L50 ([11]; [12]; [13]). Therefore, a bio-pesticides approach using essential oils with insecticidal activity has been adopted as key to the actual situation; however it is not yet known whether essential oil also influences pollinators such as bumblebee which have been currently used in tomatoes pollination by greenhouse farmers in the region of Biskra. Thus; in our study essential oil was extracted from leaves of *C. Schoenanthus* by hydro-distillation and its fumigant insecticid effect is evaluated on *Bombus terrestris* under laboratory conditions.

2. Materials and Methods

The experiment was conducted at the CRSTRA Bio-systematic laboratory on the 28th and 29th October 2018.

2.1. Vegetal material

C. schoenanthus plants were collected from the steppic region El Guettara (Djelfa, Algeria) during April. The plants were dried up for a period of one month in aired obscure place. Every two days plants were checked and turned to avoid mould.

100 g of *C. schoenanthus* dried leaves were subjected to hydro-distillation by a Clevenger device of 1000 ml flask for 4 hours at 100°C.

For experimentation needs several leaf hydro-distillation was processed under the same conditions. Extracted essential oil was diluted using distilled water.

2.2. *Insects*

The experiment was performed with 10 worker bumblebees obtained from two Agrobio colonies and conducted under standardized laboratory conditions of 24°C, 53% RH (Relative Humidity) and continuous darkness. The insects were provided with diluted commercial honey as energy source and water.

2.3. Test

The E O of *C. schoenanthus* is used in a randomized design with three replicates (10 bumblebees) to evaluate toxicity by inhalation way.

Bumblebees were first captures individually in plastic capsule, after they were submitted to 8°C temperature for 12 mn to calm them down, then in groups of 10 individuals introduced in boxes of (15 cm X 15 cm X 10 cm) containing a filter paper saturated with 3 ml of each concentration of the *C. schoenanthus* E O (0, 20 and 1000µl/ml). Choice of concentration was according to those tested on *Tuta absoluta* inthe project (unpublished data). Mortality was assessed after 4h and 24h. Preliminary tests indicated that methodology used did not effect the survival of bumblebees.

Half-maximal lethal dose (LC_{50}) values were assessed by using Probit analysis.

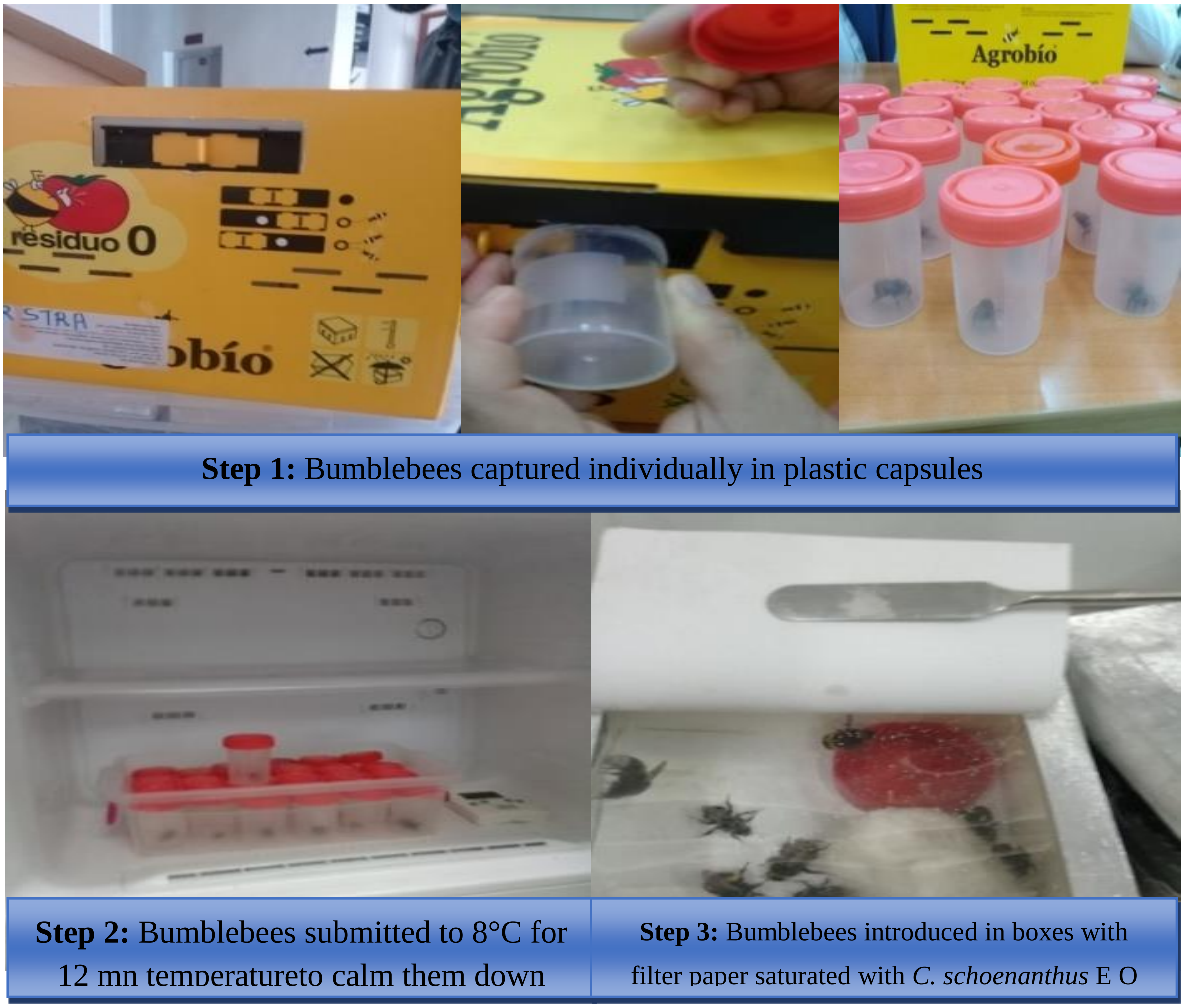


Figure 1: Different stage of *C. schoenanthus* E O. effect evaluation on *Bombusterestris*.

3. Results

Results are represented in Table 1

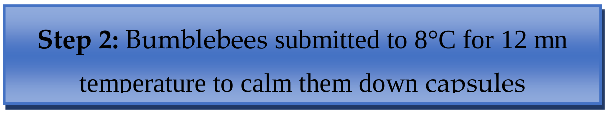
Table1. *Bombusterrestris mortality rates by C. schoenanthus essential oils in premilinary fumigant toxicity test.*

Concentration µl/ml	Mortality rate (% ± SEM) after different intervals of exposure		Mean
	4h	24h	
0	0±0.0	0±0.0	0±0.0
20	0±0.0	0±0.0	0±0.0
1000	3±0.0	96.66±0.44	53.33±0.22

(P > 0.05, n=30)

Fumigant test results showed survival function of workers over time in the control treatment and 20 µl/ml treatment. Mortality percentage 4 hours after initial exposure to 1000 µl/ml was 10%. The percent mortality 24 hours after first exposure of the bumblebee was the heighest at 96,66% ±4,44. Meanwhile a repulsive effect is observed since the first hour after the application of the E O of *C. Schoenanthus*.

Mortality regression calculated by Probit Analysis using SPSS10 reports that; the E O of *C. Schoenanthus* used in this study affected Bumblebees "*B. terestris*" in varying degrees after 24 hours (Figure2). Mortality rate increased gradually with increasing *C. Schoenanthus* Doses. Thus; LC50= 277,42 µl/ml and LC90=684,71 µl/ml.



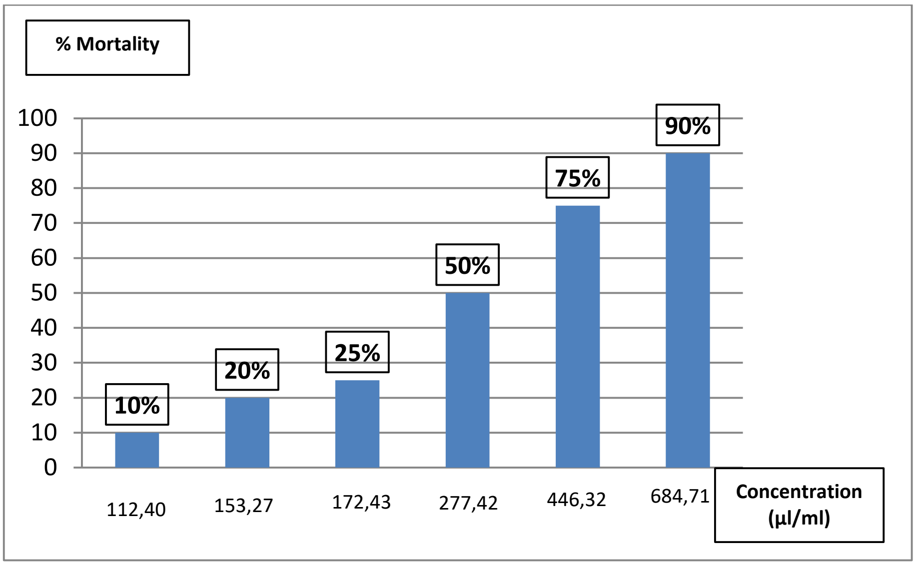


Figure2: Mortality evolution according to *C. Schoenanthus* E O concentrations.

4. Discussion

During the last years side-effects of insecticides on bees have received large interest since their value as pollinators. In Europe insecticides are tested following the EPPO (European and Mediterranean Plant Protection Organization) guiding principle to restrict any damage to honeybees *Apis mellifera*. Regrettably there was a decline in the bumblebees (*Bombus* sp., Apidae) abundance in many regioons, and it is probable that this is due in part to the application of specific pesticides [14].

In curent years, numerous researches on the pesticides' acute laboratory toxicity bumblebees were carried out [15] however little researchers worked on Bio-pesticides toxicity to bumblebees. Synthetic pesticides are amongst the principal threatening factors for wild and managed bees. In curent years, botanical biopesticides have received increasing concern and use in agriculture because of their high selectivity and short persistence in the environment [16]. Thus, [17] reported that some tested entomopathogenic fungi and entomopathogenic nematode - biopesticides may have feebly (Nematac and Nibortem) or practically poisonous (Nimbecidine, Nostalgist and Priority) outcome on *B. terrestris* workers.

We started this work by asking the question whether the essential oils of *C. schoenanthus* is safe for Bumblebees. The question is important because these essential oils are undergoing tests in laboratory for the control of *Tuta absoluta* which severly attacks tomatoes cultivated undergreenhouse conditions in the region of Biskra (Algeria). Meanwhile farmers in this region have introduced *Bombus terrestris* as tomatoes crop pollinator under greenhouses.

Our results showed that the bumblebee *B. terrestris* was not vulnerable to the 20 µl/ml, while it was most sensitive to the 1000 µl/ml concentration after 24 hours. So after 4 hours 10 bumblebees suffered 10% mortality. In spite of the dominance of the application of chemical pesticides in Algeria; we can suggest the use of the E O of *C. Schoenanthus* with concentration of 20 µl/ml or less to control pests in areas where contact with bumblebees is likely to happen especially in greenhouses where bumblebees play a vital role in pollination. While experiments with higher concentration should be performed to evaluate their effect on bumblebees as in an experiment of [18] who reported that utilization of concentrations greater than 50% of the essential oils of sweet fennel (*Foeniculum vulgare* Mill) or pignut [*Hyptis suaveolens* (L.) Poit] had been harmful for bees.

The experiment pronounced here gives supplementary evidence sustaining the adoption of conditioning techniques to asses the use of essential oils to control pests as they might be harmful for beneficial insects especially pollinating ones such as bumble-bees.

Even though the possible patterns of pesticide contact are alike for bumblebees and honey bees, the received dose may differ owed to morphological and behavioral variations. Bumblebee adults are commonly bigger than honey bees. Therefore, bumblebees show a lesser surface area to volume ratio and, for that reason, at a definite contact exposure, will get a slighter dosage of pesticide per unit body mass. Bumblebees also are protected in dense hair, which prevents pesticides from touching their cuticle. Those two essential traits indicate that exposure, and consequently the amount, through direct contact may be lesser for individual adult bumblebees compared with honey bees [19].

Therefore, considering the mortality rates obtained, we consolidate the statement of [20]; who reported that proficient use of bumblebees for crop pollination implicates respecting the necessary waiting periods before releasing them onto a treated crop in order to avoid toxic facet outcomes from pest control. Our results suggest taking adequate precautions when using bio-pesticides to ensure their effectiveness in pest control practices analogous to pollinators' safety.

5. Conclusions

Recently, the commercially Bumblebees are widely used in greenhouse crops pollination in the region of Biskra (Algeria). Meanwhile, there is recourse to the use of bio-pesticides as an alternative to synthetic pesticides and as synthesis pesticides effects on bumble bees are currently more documented, however effects of bio-pesticides on bumble-bees remain poorly investigated. This lack of assessment must be filled and standardized. Our results demonstrate that *Cymbopogon schoenanthus'* E O in small doses does not cause *Bombus terrestris* mortality but it remains to be verified that if these doses can influence the pollination behavior of bumblebees. In addition, we noted that most (but not all) of the bumblebees that had been treated with 1000 µl/ml of *C. schoenanthus*' E O died at the end of our trial, therefore, more experiments are required to test whether 1000 µl/ml of *C. schoenanthus* E O increases the mortality of foragers inside the nest via either acute or delayed lethal doses. Indeed; it is suggested that behavior tests should be integrated in risk assessment for doses superior to 20 µl/ml of *C. schoenanthus* E O because deficiency of the foraging behavior can produce in a minor pollination. Thus more laboratory tests for bumblebees are needed to evaluate the impact of bio-pesticides effects on their foraging behavior. Thus, results of this study can be helpful for further researches on assessing Bio-pesticides effects on beneficial insects precisely Bumble bees.

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