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EVALUATION OF THE EFFICACY OF DIFFERENT ACARICIDES AGAINST *VARROA DESTRUCTOR* ON A*PIS MELLIFERA INTERMISSA* IN ALGERIA

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ABSTRACT: *Varroa* mite has become a major concern of beekeepers in Algeria since the discovery of the first cases of infestation in the year 1982. The objective of this study was to test different registered chemicals aside with those prepared by beekeepers. The experiment was conducted on 50 *Apis meliffera intermissa* colonies in a commercial apiary and kept in standard Langstroth hives. Among the products which are approved in Algeria and tested in our experiment are Bayvarol which recorded the highest efficiency rate (91.62%), followed by Apivar (86.50%) and then Apistan (77.75%). Traditional preparations treatments had very low efficiency where it was only: 39.37% for amitraz and 44.21% for tau-fluvalinate (Mavrik). Our study showed a reduction in the efficiency of commercial products (Apistan, Bayvarol and Apivar) and a very low efficiency for amitraz and Mavrik. Such results prove the high demand of searching for more effective treatments against *Varroa*.

KEY WORDS: *Varroa destructor*, acaricides, honey bee, control, effectiveness

INTRODUCTION

Varroa disease is a parasitic disease of adult bees and brood, caused by an external parasitic mite, *Varroa destructor* (Anderson and Trueman 2000); it is the most important parasite of *Apis mellifera* that influences the colony development and performance (Ball 2003; Bowen-Walker and Gunn 2001), and is considered as the most serious problem of the beekeeping industry world wide (Fries 2005; Haddad et al. 2007, Guzman-Novoa et al. 2010; Di Prisco et al. 2011; Adjlane et al. 2012).

The *Varroa* mite feeds on both adult bees and brood, but reproduction is restricted to brood cells, which mites invade during the final larval developmental stage of the honey bee. Offspring is produced during the period that the immature bee develops in the capped brood cell and the mother and her progeny emerge together with the young bee (Beetsma et al. 1999). The work of Yang and Cox-Foster (2005) clearly shows that the *Varroa* weakens the immune system of bees and makes it more susceptible to viral and bacterial infections. The parasite causes deformities and weakness of the young workers. Heavy infestation causes death before the emergence of nymphs and cause the appearance of mutilated bees (Boecking and Genersch 2008). The feeding of the mite on honeybees activates the replication process in the infested bees; mites are also vectors of these virus by transmitting them from and to both adult bees and pupae (Ball and Allen 1988).

V*arroa* mite has become a major concern of beekeepers since the discovery of the first cases of infestation in the eastern areas of Algeria in 1982 in hives of honey bee *Apis mellifera intermissa,* and since that time many acaricides were used against it (Adjlane et al. 2011). Due to external factors such as climate and/or the application methods, the effectiveness of these products was fluctuating.

The phenomenon of resistance vis-à-vis several chemical molecules has been reported by several authors (Lodesani et al. 1995; Vandame et al. 1995; Londzin and Sledzinky 1996; Elzen et al. 1988; Milani and Della Vedova 2002; Garcia-Salinas et al. 2006; Semkiw et al. 2013). Only two studies where published about the effectiveness of acaricides in Algeria (Alloui et al. 2002; Loucif-Ayad et al. 2010).

The objective of this experiment was to study the efficacy of manufactured plastic strips impregnated with amitraz 500 mg (Apivar®, VETOPHARM), with flumethrin 0.06% (Bayvarol®, Bayer HealthCare) and tau-fluvalinate 0.8 g (Apistan ®, VitaEurope Limited), aside with home made strips of tau-fluvalinate and amitraz; as a common practice, these strips are introduced into the colonies and left several months (Adjlane et al. 2012).

The tested products (Apistan, Apivar and Bayvarol) are presented into the colonies according the manufacturer recommendation, These products are plastic polymer stips embedded with the active molecule. The strips should be placed in the hive, with two strips used for every 5 frames of bees in each brood chamber. The strip is hung between the

frames, with the frames separated slightly so that both sides of the strip come into contact with the bees. The strips should be removed after 6 weeks. The Mavrik acaricide ® is a milky coloured liquid, 240 mL per litter of active materials. It is an insecticide used in arboriculture. This product is impregnated into wood inserts that are introduced in the colonies with two strips per colony for 6 weeks. The amitraz molecule is applied as a few drops of a mixture to the flight entry. the application is repeated 5 times at intervals of 6 days. This study also aims to assess the effectiveness of these treatments against *Varroa* and to and to estimate the difference in honey production between 5 treatments

MATERIALS AND METHODS

The experiment was conducted in a privet apiary of a professional beekeeper in the Mitidja area in the region of Blida (36°34′59″N and 3°0′0″ E) during the period between September to December 2012. It is an about 100 km long and 15 to 20 km wide depression shut in the Tell Atlas in the south and the north Sahel, widely open to the sea with a total area of 1400 km^2 and an agricultural area from 120 000 to 130 000 hectares. This area is characterizes by big citrus orchards with a long blooming period between middle of February to early April, citrus is considered as one of the many honey harvest in the country.

Fifty *Apis mellifera intermissa* colonies kept in standard Langstroth hives previously standardized for honeybee population, brood combs and food storage were used. In each colony, a mobile bottom board was installed with wire screen to count dead mites and to avoid mite removal by honeybees. The natural mite mortality is equal for the fifty honey bee colonies. Natural mortality rate does not change the test.

Colonies were divided into 5 groups of 10 colonies each. Colonies in each group were randomly selected to receive one of five treatments:

Group 1 (T1) = treatment with Apistan®; Two strips were placed between frames close to the brood nest area for 6 weeks.

Group 2 (T2) = treatment with Bayvarol®; four strips were placed between frames close to the brood nest area for 6 weeks.

Group 3 (T3) = treatment with Apivar®; Two strips were placed between frames close to the brood nest area for 6 weeks.

Group 4 (T4) = treatment with Mavrik; Two strips were placed between frames close to the brood nest area for 6 weeks.

Group 5 (T5) = treatment with amitraz, colonies received 4 treatments of Amitraz

In order to evaluate total mite population, each group was treated with CheckMite+® at the end of each treatment for each group. CheckMite+ consists of a plastic polymer embedded with coumaphos. The strips should be placed in the hive with one strip used for every 5 frames of bees in each brood chamber. The strip is hung between the frames, with the frames separated slightly so that both sides of the strip come into contact with the bees. The strips should be removed after 6 weeks. It is important to point out that the CheckMite+® was imported for the experiment and it is not registered nor used in Algeria.

Treatment efficacy (percent efficacy, EFFIC) was calculated for each colony as follows:

$$\text{EFFIC} = \frac{\text{Number of mites dead by (T1 or T2 or T3 or T4 or T5)}}{\text{Total number of dead mites (CheckMite+®.+ T1 or T2 or T3 or T4 or T5)}} \times 100$$

After the period of citrus honey (April), a honey harvest has been completed and the amount of honey is reported for each colony.

RESULTS AND DISCUSSION

Table shows the average efficacy of all products for all colonies of five groups. Among the approved products in Algeria, Bayvarol recorded the highest (91.62%) efficacy rate, followed by Apivar (86.50%)and then Apistan (77.75%). These rates are considered very low compared to the actual therapeutic value of three products recommended by manufacturers (99%). Statistical analysis revealed a significant difference between the three products (ANOVA, $F_{(4, 45)} = 70.56$, $p<0.01$).

The home made traditional treatments had a very low efficacy where it was 39.37% for amitraz and 44.21% for Mavrik.

A clear significant difference of the mean honey production per treatment is shown in Fig. 1 (ANOVA, $F_{(4, 45)} = 60.40$, $p<0.01$). Whereas the home made traditional treatments had the lowest honey production average.

Loucif Ayad et al (2010) reported in trials conducted in eastern Algeria an efficacy of 89% for Bayvarol and 85% for Apivar. Alloui et al. (2002) have found 99.1% efficacy using flumethrin soaked stripes on November in Algeria, at the end of six weeks. Trials about another commercial product which contains flumethrin were performed in Turkey and detected 87.7% and

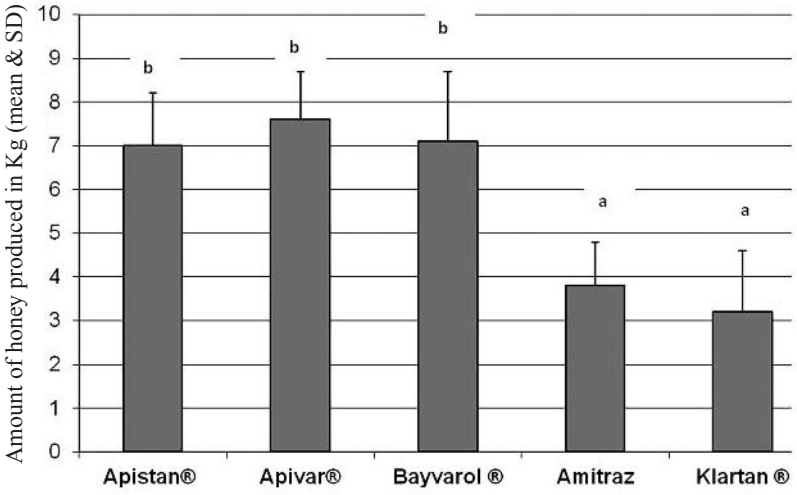

Fig 1. The mean amount of honey produced from colonies treated with different treatments (letters a, b indicate significant differences between treatments, P <0.01)

Table.
The efficacy of the five experimental groups (letters a, b, c and d indicates significant differences between treatments, P <0.01)

Apistan®		Apivar®		Bayvarol ®		Amitraz		Mavrik ®	
Number of colony	Efficacy (%)	Number of colony	Efficacy (%)	Number of colony	Efficacy (%)	Number of colony	Efficacy (%)	Number of colony	Efficacy (%)
1	65.55	11	88.52	21	91.1	31	45.34	41	32.52
2	71.68	12	91.84	22	89.52	32	39.52	42	49.55
3	88.95	13	85.92	23	81.52	33	47.98	43	66.87
4	75.95	14	92.45	24	94.55	34	33.89	44	58.52
5	82.31	15	81.53	25	88.56	35	23.23	45	61.25
6	86.74	16	79.54	26	82.69	36	57.21	46	33.25
7	66.75	17	84.22	27	95.45	37	39.41	47	29.58
8	58.23	18	86.78	28	89.74	38	44.98	48	41.22
9	89.56	19	81.74	29	94.58	39	40.5	49	29.85
10	91.77	20	92.55	30	93.45	40	39.34	50	39.55
Average ± standard deviation	77.75 b ± 11.81	Average ± standard deviation	86.50 c ± 4.78	Average ± standard deviation	91.62 d ± 4.17	Average ± standard deviation	39.37 a ± 6.93	Average ± standard deviation	44.216 a ± 13.92

100.0% efficacies in broodless period in fall season (Akkaya and Vurusaner 1996, 1997). In Slovenia, Jelinski (1993) reported an efficacy of 59.6% for Bayvarol.

Floris et al. (2001) have found 75% efficacy using amitraz in Italy. The effectiveness of five products: Apistan, Bayvarol, Apivar, Perizine and Bee Strips against *Varroa destructor* was evaluated during three successive seasons (October – December, 2003, 2004 and 2005) by Al-ghamdi (2007) in Saudi Arabia. The results revealed a decline in the efficacy of Apistan and Bayvarol, which was attributed to the development of resistance in *V. destructor* against fluvalinate and flumethrin, while the Apivar was proved still the most effective acaricide.

Gregorc and Skerl (2007) have found 94.3% efficacy in highly infested colonies and 19.11% in slightly infested colonies after 40 days flumethrin treatment in Slovenia. In France, Faucon et al. (2007) conclude that Apivar ND preserved treated colonies against 99,5% of the population found in control colonies. The tests carried out in Ghazvin in Iran against *Varroa destructor* showed a good effectiveness, of Apivar® 96.68%, for Bayvarol® 96.59% (Shahrouzi 2009). Flumethrin treatment demonstrated insufficient efficacy for mite reduction in Slovenia, being approximately 73% in

2007, but only 13% in 2008 (Љkerl et al. 2011). The efficacy of Apistan was 81.7% in Lithuania (Pileckas et al. 2011). The average efficacy of amitraz calculated for the two years combined, after 6 and 8 weeks of treatment, amounted to 90.6% and 94.6%, respectively (Semkiw et al. 2013).

Our results indicate a possible occurrence of *Varroa* resistance to approved chemicals. The phenomenon of *Varroa* resistance to Fluvalinate was reported for the first time in Italy in 1994 (Lodesani et al. 1995). Also in Italy, more tests have detected *Varroa* resistance to bromopropylate and coumaphos (Milani 1999; Spreafico et al. 2001). In the United States, the first centre of resistance symptoms was discovered in 1997 (Elzen et al. 1998). Also in the United States, Elzen and Westervelt (2002) reported the first case of resistance of *Varroa* to coumaphos in Florida after only 4 years of use as a *Varroa* treatment. *Varroa destructor* resistance to amitraz has been described by several authors (Elzen et al. 1999; Mathieu and Falcon 2000; Rodriguez-Dehaibes et al. 2005).

In *Varroa* population control, acaricide rotation is widely accepted to mitigate resistance development (Faucon et al. 2005). According to Sammataro et al. (2005), the presence of resistant mites (in colonies where no strong acaricides pressures are obvious) may be due to (1) bees robbing honey from a weak or dying hive (with resistant mites) within the flight range of the apiary and, as a consequence, acquiring those mites; (2) introduction of packaged bees and queens from other states already parasitized by resistant mites; or (3) drifting bees, a common phenomenon in large apiaries where phoretic mites can be swiftly distributed throughout the whole apiary in a short time.

Many cases of resistance in Europe were associated with the use of agricultural formulations of pyrethroids (Watkins 1997) or high use of varroacide strips which significantly increase the selection pressure for resistant mites (Milani 1999).

In the United States, in addition to the molecule Fluvalinate, other research reports show that the use of amitraz in the fight against *Varroa* is ineffective (Elzen et al. 1999). Thus, formic acid gel (Apicure®) and the organophosphate insecticide coumaphos received authorization on the market to fight against *Varroa* resistant populations (Feldlaufer et al. 1997; Kochansky 2000). In addition, the cross product is very useful in the fight against *Varroa*, it prevents the emergence of varroa mites that are 100% resistant against the Fluvalinate. Trouiller (1997) reported that resistance may disappear naturally and spontaneously through reversion way. The same author relates that in an experiment conducted in an isolated apiary resistance rate in a colony can grow from 50 to 16% in one year.

Milani and Della Vedova (2002) studied the reversion of resistance to pyrethroids in *Varroa* by monitoring the decrease in the proportion of resistant mites in southern Italy. These authors concluded from their tests, which lasted three years (1997 to 2000), that the influence of the reversion of selecting resistant mites is negligible if the treatments take place each year. Still according to the same authors, in areas where the resistant mites are present, treatments against *Varroa* can only be effective if they are applied at intervals of several years.

CONCLUSION

A practical recommendation is coming out of this research project, where it is clear that the efficacy of preparations of amitraz and fluvulinate is very low, and it is highly recommended that the beekeepers of Algeria should not any longer use the treatment methods they are practising. Moreover, these methods cause a decrease in the honey production. It is essential to point out that even the official registered products are showing a reduction in their efficacy, which requires further test and search for effective *Varroa* treatments.

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