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Effect of Different Concentrations of M2I™ Pheromone Dispensers and the Impact of Water and Paraffin in Pheromone Traps for *Rhynchophorus Ferrugineus* (Coleoptera: Curculionidae) Management in Tunisia

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Abstract **– Red palm weevil is considered nowadays one of the major threats to palm trees worldwide.**

Due to the current issue towards the use of environmentally friendly control measures, dealing with this pest by mass trapping was used in this study. In order to enhance the current management practices to control the pest, field studies were conducted to know the attractiveness of red palm weevil to 3 concentrations of polymers in M2I™ dispensers in comparison to ISCA capsules, and to test the effectiveness of putting water inside the traps in the adults' attraction. Besides we tested the impact of three solutions: bare paraffin, paraffin mixed with water and bare water in order to evaluate the best solution that attract more adults of Red palm weevil. Studies indicated that the concentration 0.5 % and 1% (of polymers) attracted significantly more number of weevils. Studies revealed also that there wasn't significance in putting water inside traps. The solutions tested inside the traps proved that paraffin is the best substance that bans more adults to escape from trap. This study indicated the existence of red palm weevil throughout the year but with lower adults capture in the study period (October-December). We presented climatic data in details mainly temperature and Relative humidity. These parameters were main explication for the lower rate of RPW adults captured in baited traps. Pheromone traps baited with M2I dispensers can be placed in fields for better IPM for RPW.

Keywords **– *Rhynchophorus Ferrugineus*, Capsules M2I™, Water, Traps.**

I. INTRODUCTION

Rhynchophorus ferrugineus (Olivier) (Coleoptera Dryophthoridae) is a serious pest inflicting severe damage to date palms.

The chemical control of this pest has been discouraging, since it leads to the pollution of water sources around application areas [1] - [2] - [3]. Preventive and curative methods were often based on chemical pesticides. [4]

During the last decade multiple introductions of *R.ferrugineus* have occurred to the Middle East reaching countries of North Africa. It attacks a wide range of ornamental palms. [5]

The latest report of an RPW invasion occurred in late 2011 in Tunisia where it was found infesting *Phoenix canariensis* [6]. On this last species the weevil's infestation occurs on the top of the tree. Such an attack pattern is due to the massive pest presence throughout the whole year, the severe damage occurring within infested trees and the late onset of the symptoms' expression make their detection difficult. The detection of the palm borers essentially relies on visual search of symptoms that have to be effectively recognized. This task is very laborious and requires an expertise because it requests access to the foliage for the tall palm specimens. [7]

Four stages were defined, revealing the outcome of the attack on the leaves and the crown of *P. canariensis*. Stage 0 defined as an asymptotic palm. Stage 1 is characterized by some chewing symptoms in inner leaves, Second Stage is marked by Extensive chewing symptoms of ">" shape. Asymmetric inner leaf growth is clear in Stage 3 of an attack and on the last stage all the crown leaves collapsed into an "umbrella" shape. As the eggs of RPW are deposited inside concealed places of the stem, larvae hatch and start destructing reaching generally the apical growth area. [8] and [9].

Due to the current trend towards the use of environmentally-friendly control measures, increasing interest is being shown in trapping as a way of dealing with this pest [10] – [11]-[12].

To reduce the risk of environmental pollution, therefore, effort is needed to develop for safer and more effective chemicals application methods.

The M2i dispensers used for this study are based on a new patented technology of formulation that can release simultaneously the highly volatile kairomone and the poorly volatile pheromone (ferrugineol) thanks to the use of a specific polymer. [13] – [14]-[15]

The present study assessed the influence of 3 concentrations of polymers from M2I™ on the capture of *R. ferrugineus* adults and identified possible reasons for the better performance of pheromone traps. Two tests were carried out using colored bucket traps; in the first, the traps were baited with male aggregation pheromone using dispensers of M2I™ with 3 provided concentrations 0.5%, 1% and 2% of polymers. While the second involved traps.

The aim of the present study was to analyze the importance of the olfactive factor in captures of *R. ferrugineus* adults. In order to confirm the effect of olfactive attraction on the number of captures, tests were carried out on the attractiveness of traps baited with three types of concentrations compared to ISCA capsules. The efficacy of two models of traps was also assessed in

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relation to internal trap climatic conditions (temperature and relative humidity), *R. ferrugineus* adults.

The results obtained provide information that should be helpful in improving the control of *R. ferrugineus* by mass trapping systems.

Fig. 1. Palms attacked with RPW in experimental sites.

II. Materials and Methods

A. Study Sites

Experiments were conducted in RPW infested date plantations in Tunis, Tunisia for 3 months from October to December; 2017. The choice of experimental sites was essentially based on the infestation symptoms witnessed on palms. We restricted our choice on protected area in order to avoid the damage of traps. In the first part we tested the effect of 3 concentrations of polymers in M2I dispensers in capturing adults.

a) The Test of the Concentrations in Capturing RPW Adults

The different polymers' concentrations tested were 0.5; 1 and 2% in comparison to ISCA capsules.

Trap characteristics in both tests the traps consisted of: (1) a 10 liter plastic black bucket, with four 6 × 4 cm holes cut below the upper rim, at a distance of 20 cm from the base; (2) a funnel, placed inside the bucket to prevent the weevils escaping; (3) a lid for the bucket, with four holes similar to those in the bucket, with a piece of steel wire to hang the chemical attractants on (pheromone and kairomone dispensers (Fig. 3.B). We used as well M2I yellow traps (Fig. 3.A). The traps baited with olfactory attractants at different concentrations were tested in 2 Palm tree groves highly infested by *R. ferrugineus*, near the town of Tunis. From beginning of October to end of December 2017. They contained: (1) a R. ferrugineus male aggregation pheromone dispenser (Rhynchoclassic® Pheromone, M2I, France). The pheromone dispensers had a usable life of 3 months. The comparison of these formulations to the ISCA capsules would determine the best formula that captures the maximum of RPW adults in Tunisian climate conditions.

The traps 'combinations were prepared as indicated in figure 2.

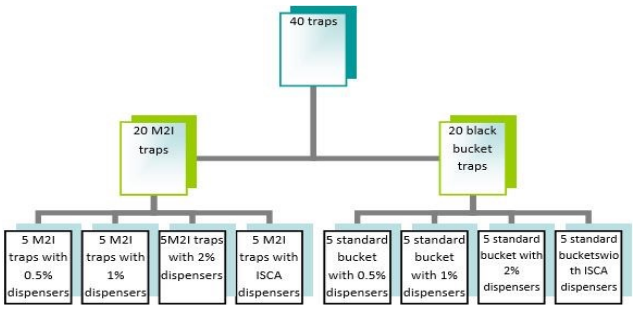

Fig. 2. Eight combinations of red palm weevil aggregation pheromone trap tested in the study.

The traps were placed on the ground, as according to Oehlschlager (2006), in this position, the insect have a better landing area, and consequently the traps capture more weevils. The traps were installed randomly; we recorded every attacked palm tree as well as traps by GPS points. The essay was repeated in two sites, the first one was the national institute of agronomy of Tunis and the second site was the public zoo park Belvedere.

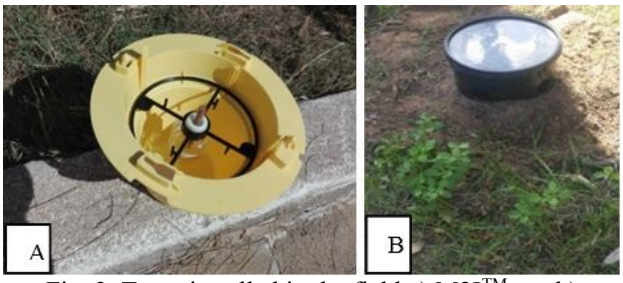

Fig. 3. Traps installed in the field a) M2I^{TM}trap b) Standard bucket trap.

The number of adults captured in each trap was recorded each week with the climate conditions (temperature and relative humidity).

1 -*National Institute of Agronomy Tunis (INAT)*

Characteristics:

In this site we counted 78 ornamental palm trees, 11 palm trees were infested in the first stage.3 palm trees were infested in the second stage and one palm tree in the fourth stage.

Climate Conditions:

In each control date we recorded relative humidity (Fig. 6). Temperature variations were obtained from accuclimate site, 2017 as it is represented in figures (4, 5, 6 and 7).

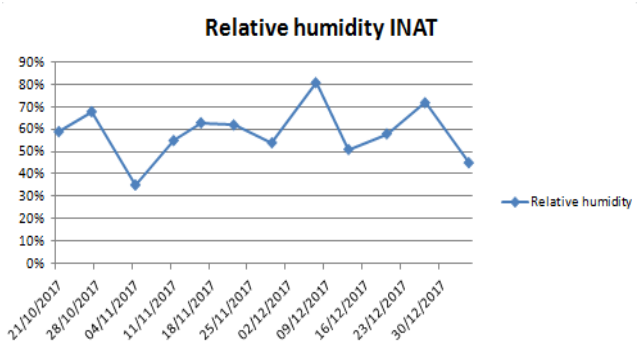

Fig. 4. Relative humidity in site1 INAT.

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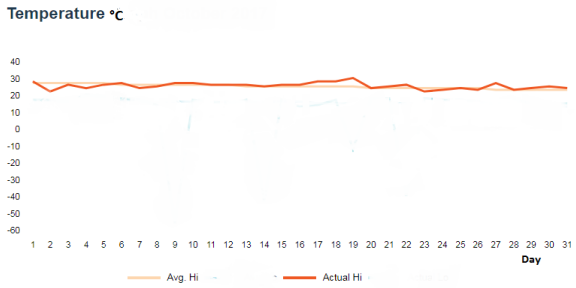

Fig. 5. Temperature variations during the month of October in site 1 (INAT, Tunis).

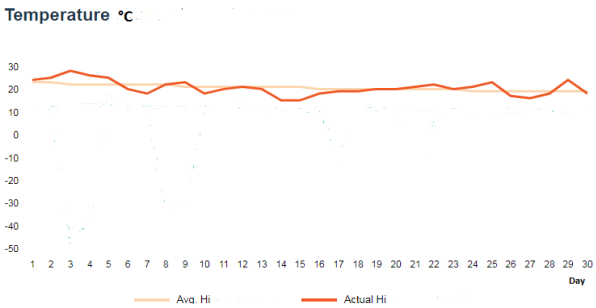

Fig. 6. Temperature variations during the month of November in site 1 (INAT, Tunis).

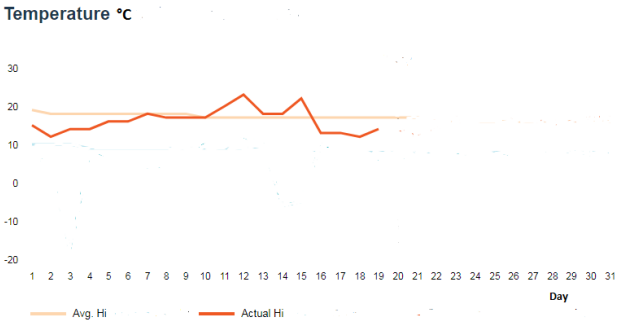

Fig. 7. Temperature variations during the month of December in site 1 (INAT, Tunis).

2-*The Public Zoo Park "Belvedere"*

Characteristics

51 palm trees were recorder in Belvedere zoo park.5 palm trees were in first stage, 2 palm trees in third stage. We have to mention that this site is routinely controlled and the palm trees are treated with insecticides.

Climate conditions

Temperature variations in site 2 (Belvedere zoo Park) during 3 months are revealed in figure 8, 9 and 10

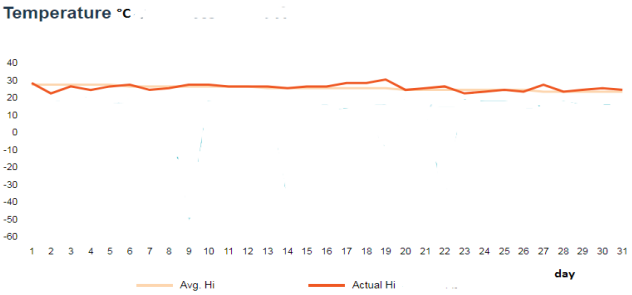

Fig. 8. Temperature variations during the month of October in site 2 (Belvedere, Tunis).

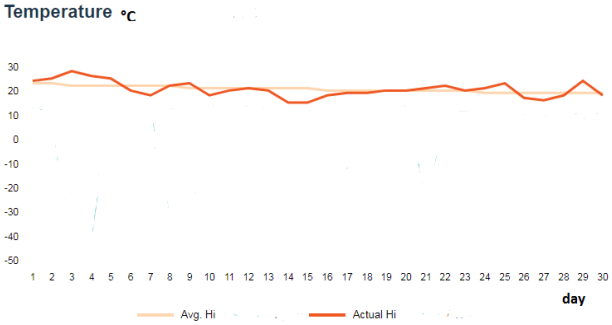

Fig. 9. Temperature variations during the month of November in site 2 (Belvedere, Tunis).

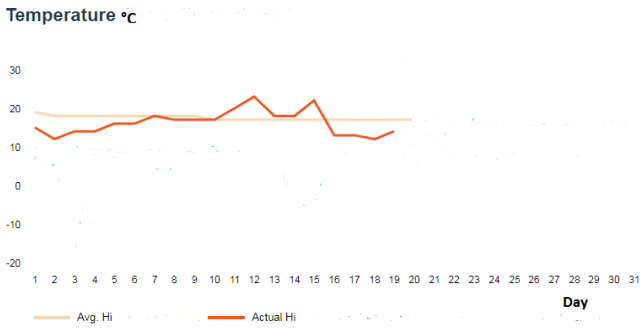

Fig. 10. Temperature variations during the month of December in site 2 (Belvedere, Tunis).

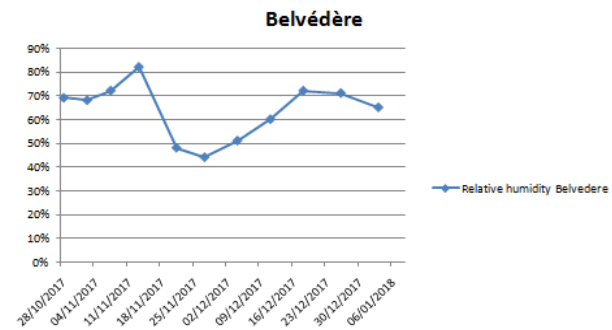

Fig. 11. Relative humidity registered in site 2 Belvedere.

b) The Effect of Water in Capturing Adults

In this essay we only used 22ml dispensers covering 0.5% of polymer.

This essay was repeated in two sites: The residence of French embassy in La Marsa and youth house in Rades. We used two types of pheromone traps. The M2I model and the standard black bucket as described by [16] and [17] with 4 windows. The pheromone dispenser was put in the middle of each trap. Then we buried the pheromone traps into the soil. This would facilitate the pest penetration in the trap.

In this essay we used 22ml dispensers covering 0.5% of polymer.

We established 40 traps: 20 M2I traps and twenty standard black traps. The half amount of traps was filled with water in order to prevent

Prevent the escape of the adults. The rest of the quantity doesn't contain water. The traps were inspected weekly during the experimental period.

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We installed the traps randomly and alternately respecting 20 m distance between a trap and another. In order to prevent the escape of pests.

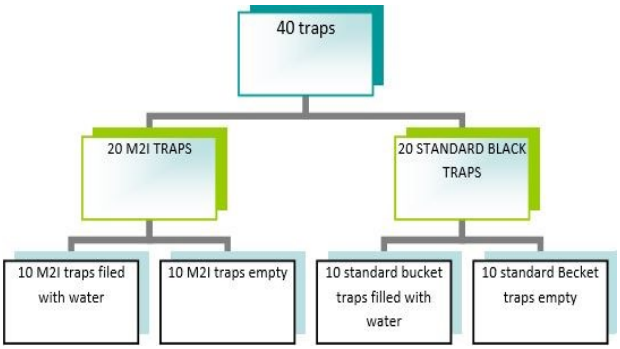

Fig. 12. Combinations of red palm weevil aggregation pheromone trap tested in the study.

1- Site 3 Residence of French embassy

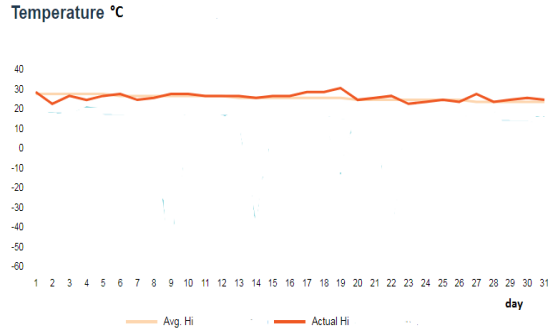

Fig. 13. Temperature variations during the month of October in site 3 (French embassy, Tunis).

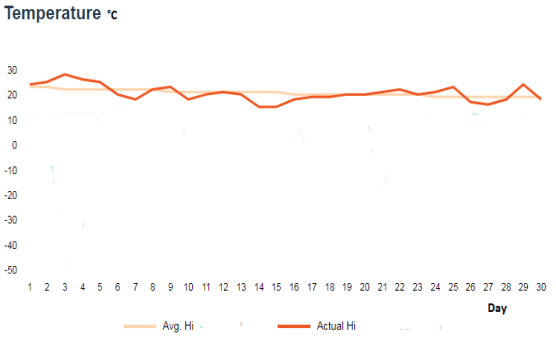

Fig. 14. Temperature variations during the month of November in site 3 (French embassy, La marsa).

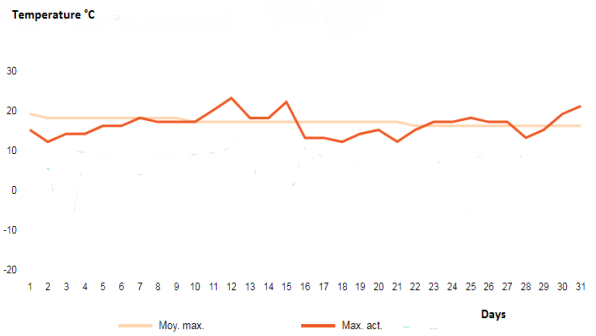

Fig. 15. Temperature variations during the month of December in site 3 (French embassy, La marsa)

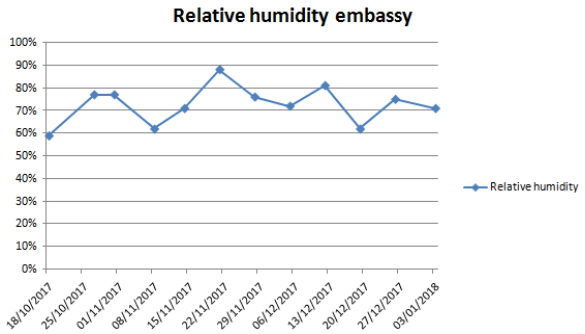

Fig. 16. Relative humidity registered in the embassy of France, la marsa.

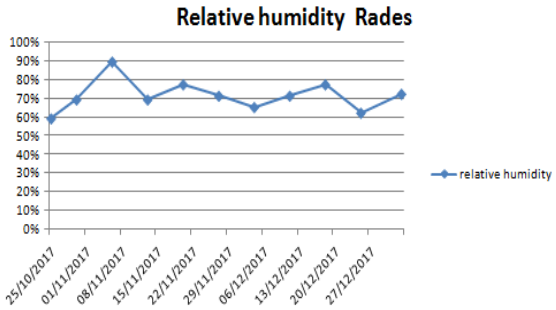

Fig.17. Relative humidity registered in Rades

C) The Effect of Solutions Chosen Inside the Trap

This essay was conducted in 2 sites in order to determine the effect of the solution put inside the trap in capturing RPW adults. This essay would conclude whether the paraffin (M2I^{TM}) is better in banning adults to escape from traps in comparison with water.

Thirty M2I traps were installed in two sites. Each ten traps contained one of the following solutions: Paraffin, water, and paraffin with water. The quantity put inside was 150ml. For the traps, where we put water and paraffin, we put 75ml of water mixed with 75ml of paraffin.

Each week we recorded the adults captured in the traps.

Fig. 18. Captured RPW adults in M2I trap

d) Statistical analysis:

The data were subjected to ANOVA and the means were compared by carrying out the Least Significant Difference test (LSD 5%).We used SASS and SPSS (20) for statistical analysis.

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III. Results and Discussion

a) The Test of the Concentrations in Capturing RPW Adults

Table 1. Testing effects of Time, trap type and capsule concentration in site 1: INAT

Score Statistics For Type 3 GEE Analysis

Source	DF	Chi-Square	Pr > ChiSq
Time	10	31.40	0.0005
Trap model	1	0.69	0.4055
Concentration in Polymer.	3	13.61	0.0035

Table 2. ANOVA for the effect of different concentrations of polymers on the captures of the red palm weevil in food-baited pheromone traps in site 1: INAT

Least Squares Means

Concentration.	Estimate	Standard Error	z Value	Pr > |z|	Mean	Standard Error of Mean
0.5	-0.9353	0.1865	-5.01	<.0001	0.3925	0.07320
1	-0.8750	0.2030	-4.31	<.0001	0.4169	0.08463
2	-1.6164	0.1735	-9.32	<.0001	0.1986	0.03445
ISCA	-1.9669	0.1887	-10.42	<.0001	0.1399	0.02640

Based on previous results [17] done during the spring. These results indicated that red palm weevil was presented all over the year, and increases the damage, infestation severity. The table 1 shows that trapping adults in different traps is affected mainly with time (period of time starting from the date of installing traps and the date of control) the number of adults is as well affected with the trap type and dispensers' concentrations.

Table 3. Testing effects of Time, trap type and capsule concentration in site 1: INAT.

Score Statistics For Type 3 GEE Analysis

Source	DF	Chi-Square	Pr > ChiSq
Time	10	31.40	0.0005
Trap model	1	0.69	0.4055
Concentration Polymer.	3	13.61	0.0035

Score Statistics For Type 3 GEE Analysis

Source	DF	Chi-Square	Pr > ChiSq
Time	10	31.40	0.0005
Trap model	1	0.69	0.4055
Concentration Polymer.	3	13.61	0.0035

Score Statistics For Type 3 GEE Analysis

Source	DF	Chi-Square	Pr > ChiSq
Concentration Polymer.	3	13.61	0.0035

Table 4. ANOVA for the effect of different concentrations of polymers on the captures of the red palm weevil in food-baited pheromone in Belvedere (site2)

Least Squares Means

Concent.	Estimate	Standard Error	z Value	Pr > |z|	Mean	Standard Error of Mean
0.5%	0.4962	0.1257	3.95	<.0001	1.6424	0.2065
1%	0.5750	0.1258	4.57	<.0001	1.7771	0.2236
2%	0.2143	0.1444	1.48	0.1377	1.2390	0.1789
ISCA	-0.04013	0.2366	-0.17	0.8653	0.9607	0.2273

During studying period, Captures registered per week in different traps was low in both sites (1 and2).The number of RPW adults vary between 0 and 10,presenting a general mean of 0.43 ± 0.99.The negative binomial model revealed that there is significance of the concentration of polymers (p = 0.0035) equally the time. The trap type is not significant. It means that using M2I traps or black bucket standard traps is the same during this study period (October-December).

Results showed also that there was any interaction between trap and capsule used.

b) The Effect of Water in Capturing Adults

Table 5. CHI squared test

Chi-squared TEST

	Value	ddl	Signification
Chi-squared of Pearson	11,302[a]	7	0,126
Rapport de vraisemblance	11,912	7	0,103
Number of observations	960		

8 cells (50, 0%) have theorotical number less than 5. L'effectif théorique minimum est de ,50.

The chi-squared test, between total capture adults and the existence or not of water inside the trap (table 4), revealed that during the studying period (winter), the presence of water in not significant compared to capture results (p=0.126>0.05).

This result is explained by the lower number of adults captured during the studying essay (October-December). In fact the peak of RPW activity is highly recorded in spring and summer time [11] and [12].

C) The Effect of Solutions Chosen Inside the Trap

Comparing the mean number of adults caught in traps filled with three solutions, Results have indicated that bare paraffin filled in RPW traps registered the best weevil captures (45%) followed by traps filled with water and paraffin (32.6%).In third place traps filled only with bare water captures RPW adults with a rate of 22.4%.

IV. Conclusion

Based on the results presented and discussed above, we suggest that using either standard black-bucket RPW pheromone traps or M2I traps doesn't affect the RPW adults' capture .Pheromone traps along with 0.5%and1% dispensers would significantly enhance the efficiency of the mass-trapping programme of RPW in Tunisian climate. Hence, M2i capsules could be incorporated in RPW pheromone traps during the pest activity during the year under the environmental conditions prevailing in TUNISIA and also in and around plantations where weevil activity is high.

Further essays are recommended to confirm whether the concentrations mentioned before (0.5% and 1%) are as well efficient as it was revealed during this study.

Traps' water content is not essential during this period. Putting water or not in traps does not affect adults' capture. However putting bare paraffin in traps enhances the capture of RPW adults.

Acknowledgment

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