Egypt. J. Bot., **Vol. 58**, No. 2, pp. 145 - 156 (2018)

Prospects of Bio-pesticides for the Future in Pest Management

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IN THIS STUDY, three plant oils (fenugreek, ginseng and marjoram) and the bio-insecticide abamectin were used as agricultural insecticides. Effects of these materials on cowpea weevil (*Callosobruchus maculatus*) and on *Vicia faba* plant cells were examined. Results showed that the mortality percentage of *C. maculatus* adult increased with the increase of dose treatments (ml/kg) and by increasing the time of exposure. The residual activity of the tested materials on cowpea seeds at storage periods (3 months) showed that $2LC_{95}$ of tested oils have a reduction effect on *Callosobruchus maculatus* progeny (F_1); which decreased gradually till the 6th week while, LC_{95} of abamectin decrease the number of F_1 to zero (100% reduction) in all times of treatment until storage periods. The percentage of *C. maculatus* emergence in cowpea seed treated with the tested mixtures (oils- abamectin) showed that mixture 2 is the most effective which recorded 100% at the initial time up to 12th week.

On the other hand, the cytotoxic effects using root tips of *Vicia faba* assay showed reduction in MI in all treatments applied which increased with the increase of the concentration. It recorded 2.42, 2.01, 1.64 and 0.91% in roots treated with the highest concentration of fenugreek, ginseng, marjoram and abamectin respectively, as compared with negative control (4.18%) or with the positive control (1.83%). All the mixtures (oils- abamectin) applied recorded significant decrease in MI values. Different types of chromosome abnormalities were recorded such as stickiness, disturbance in metaphase and anaphase, C-metaphase, chromosome bridges, lagging chromosome and micronuclei at interphase.

Keywords: Botanical pesticides, Bio-insecticide, *Callosobruchus maculatus* pest, Mitotic index, Chromosomal aberration, Storage cowpea.

Introduction

Seed considers the basic and crucial input for agricultural production. Maintenance of high seed quality from harvest until planting is of almost importance in a seed production programme. During storage, the quality of seeds gets deteriorated in a number of ways of which infestation by the storage pests contribute a bulk share. Of at least one million insect species worldwide, 10000 species are crop-feeders. Among them, about 700 species cause damage to agriculture, comprising stored products (Shaaya et al., 1997 and Ware & Whitacre, 2004). *Callosobruchus maculatus* weevil is an important agricultural pest inset, has drawn attention because it is a major pest of economically leguminous seeds which resulted in 100% loss of stored cowpea (Reuben et al., 2006 and Tiroesele et al., 2015). Seed weight loss was associated with larvae of *Callosobruchus maculatus* which bores into the seed and by the time it consumes the seed cotyledons (Kshirsagar, 2010). Although various synthetic insecticides have been developed over the years for the control of pest inset, the cost of purchase, residual effect, high mammalian toxicity and the widespread development of resistance in insect pests are still issues of great attention (Udo, 2011). Due to these problems, the research has been shifted towards using natural products in crop protection (Tiroesele et al., 2015).

Many plant extracts or microorganism products have been established to possess insecticidal

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DOI: 10.21608/ejbo.2018.1994.1136

properties against a wide range of insect pests (Tiroesele et al., 2015). Attention has been given to the control of storage pests using various oils as protectants, including vegetable oils, essential oils and mineral oils because they constitute a wealthy source of bioactive chemicals (Reuben et al., 2006; Fatiha et al., 2014 and Tiroesele et al., 2015). Plant oils are readily biodegradable and less detrimental to non-target organisms than synthetic pesticides (Reuben et al., 2006). Also, different compounds separated from microorganisms such as abamectin which was isolated from *Streptomyces avermitilis* have been documented as the major source in bio-pesticide industry (Shi, 2000).

Many authors reported that higher plants bioassays are helpful in screening the bioactivity of different materials at large scale, particularly in areas with limited funds (Grant, 1994 and Grant & Owens, 2006). The effects of diverse chemicals can be spotted at the level of chromosomes through alterations in chromosome structure and number. Plant systems *in vivo*, are validated by the similar results performed in animal or human testing *in vitro* (Konuk et al., 2007 and Khalifa et al., 2015). In view of the economic importance of leguminous seeds and the intensity of damage associated with the use of synthetic insecticides (Ware & Whitacre, 2004 and Al-Ahmadi, 2013); the present work was designed to evaluate the toxicological effects of bio-insecticide abamectin and three plant oils extracted from; T*rigonella foenum-graecum*, *Panax ginseng* and *Origanum majorana* on cowpea weevil (*Callosobruchus maculatus*) and also to estimate the cytogenetic effect of these materials on *Vicia faba* plant cells.

Materials and Methods

Seeds of *Vigna unguiculata* L. (cowpea, cultivar Teba), *Vicia faba* (var. Giza2), bean beetles (*Callosobruchus maculatus*) and the bio-insecticide abamectin used in this study, were provided from the Agricultural Research Center, Giza, Egypt. Abamectin is a mixture of 80% avermectin B_{1a} and 20% avermectin B_{1b}; derived from Streptomyces avermitilis (Fisher & Mrozik, 1989). The plant oils of fenugreek (family Fabaceae), ginseng (family Araliaceae) and marjoram (family Lamiaceae) were obtained from Cap-Pharm Company; Cairo Egypt. All treatments were carried out at laboratory conditions; 27±3˚C and 65±5% RH (relative humidity).

Toxicological effects of the tested materials on cowpea weevil (Callosobruchus maculatus)

Mortality percentages of C. maculatus adults

One ml of each oil was diluted with 10ml of petroleum ether, then serious of gradual concentrations from each of tested oil and abamectin were used to estimate the mortality percentages of *C. maculatus* adults. A sample of 10g of disinfected cowpea seeds was placed in a glass tube and separately mixed with the different concentration. Three replicates for every treatment were infested by 25 adults of the tested insect. Percentages of mortality were taken after 1, 2, 3 and 5 days from exposure and calculated according to Abbott´s (1925) formula:

$$\text{Mortality (\%)} = 100\ (X–Y)/\ (100\text{-}Y)$$

where, X = Percentage of observed mortality (treatment) and Y = Percentage of observed mortality (control).

Effect of the tested materials on C. maculatus progeny (F1) at storage periods (3 months)

The concentrations of mortality regression lines for tested materials against *C. maculatus* were designed according to Finney (1971). The slope values of established lines, LC_{25}, LC_{50}, LC_{75}, LC_{90}, LC_{95} and LC_{99} were estimated after 24 h from insect exposure. LC_{20} and LC_{95} for each natural tested material were determined; then three mixtures were prepared as follows: Mixture 1= LC_{20} abamectin - 2 LC_{95} fenugreek oil; Mixture 2 = LC_{20} abamectin - 2 LC_{95} ginseng oil and Mixture 3 = LC_{20} abamectin - 2LC_{95} marjoram oil.

To study the residual effect of the tested materials, 500gm of cowpea seeds were treated with LC_{95} and 2LC_{95} of each tested oil, LC_{95} abamectin and the mixtures 1, 2, 3. Every 2 weeks twenty-five *C. maculatus* adults were placed to 10gm of the previous treated cowpea seeds and the untreated control. Three replicates were carried out for every treatment. Mortality of *C. maculatus* adults were carried out periodicalyaftertwo weeks (2^{nd}, 4^{th} and 6^{th} week….etc) till 3 months.

Effect of the tested materials and mixtures on cowpea seeds germination

Ten g of cowpea seeds were treated with 2LC_{95} of each tested oil, LC_{95} abamectin and their mixtures. The germinated seeds were recorded

and percentage of germination was calculated at initial time and after the storage period according to the formula:

Germination % = C – T/ C X100

where C = Number of germinated seeds in control and T= Number of germinated treated seeds.

Cytological studies

Seeds of *Vicia faba* allowed germinating in tap water. When the roots reached 2–3cm long, they were treated for 24 h with LC_{50}, LC_{95}, 2 LC_{95} of each tested oil; LC_{20}, LC_{50}, LC_{95} of abamectin and the mixture 1, 2, 3. All treatments were made in three replicates. Negative (H_2O) and positive (petroleum ether) control are used. Roots were fixed in Carnoy solution (3 absolute alcohols:1 glacial acetic acid) for 24 h; then roots maintained in 70% alcohol. Hydrolysis was performed in 1N HCL at 58°C for about 8 min. Root tips were stained with leucobasic Fuchsin according to Darlington & La Cour (1976). Light green dye (0.3%) was used for staining the protoplasm. Preparations were made then the frequencies of mitotic index as well as the frequencies of different mitotic abnormalities were determined. They were statistically analyzed using *t* test.

Results

Toxicological effects of the tested materials on cowpea weevil (Callosobruchus maculatus)

Mortality percentages of Callosobruchus maculatus

The susceptibility tests of cowpea weevil adults to the tested materials showed that the mortality percentage increased with the increase of dose treatments (ml/kg) and by increasing the time of exposure (Table 1). Mortality percentage was recorded 100% after 5 days from treatment with 5ml/kg fenugreek and with 5 and 4.5ml/kg of ginseng and marjoram, respectively after 2 days of exposure. On the other hand, the bio-insecticide abamectin showed 100% mortality from the second day of exposure with 0.1ml/kg; i.e. there is a potential linear relationship between the dose of treatments and mortality percentages.

Effect of the tested materials on C. maculatus progeny (F1) at storage periods (3 months)

Table 2 showed the value of LC_{25}, LC_{50}, LC_{75}, LC_{90}, LC_{95} and LC_{99} after one day from cowpea weevil insect exposure with the test materials.

TABLE 1. Mortality percentage of cowpea weevil adults after different treatments with the tested materials.

Tested materials	Dose of treatments ml/kg	Mortality % after indicated periods (days)			
		1	2	3	5
Fenugreek	2.0	25.53	33.67	41.73	58.61
	3.0	38.03	44.71	61.73	73.91
	4.0	54.61	62.58	78,38	87.33
	5.0	78.72	89.34	96.83	100
	6.0	90.10	98.72	100	100
Ginseng	1.0	19.15	27.42	43.24	60.33
	2.0	24.82	31.73	55.61	71.4
	3.0	27.66	41.28	67.82	80.13
	4.0	65.96	77.33	89.23	95.82
	5.0	97.16	100	100	100
Marjoram	1.5	21.28	32.62	46,35	61.43
	2.0	30.5	40.13	53.61	69.83
	3.0	51.77	70.02	87.52	96.67
	3.5	78.72	88.31	96.84	100
	4.5	95.74	100	100	100
Abamectin	0.003	20	37.92	51.33	69.02
	0.01	38.66	60.17	88.66	98.99
	0.02	69.33	82.88	95.33	100
	0.03	78.66	90.63	98.72	100
	0.1	97.33	100	100	100

TABLE 2. Toxicological evaluation for the test materials after one days of exposure against cowpea weevil adults.

Tested materials	LC_{25} ml/kg	LC_{50} ml/kg	LC_{75} ml/kg	LC_{90} ml/kg	LC_{95} ml/kg	LC_{99} ml/kg
Fenugreek oil	2.25	3.30	4.86	6.88	8.48	12.52
Ginseng oil	1.68	2.81	4.71	7.49	9.89	16.67
Marjoram oil	1.87	2.06	3.06	4.83	5.76	8.01
Abamectin	----	0.01	0.03	0.06	0.09	0.2

The residual activity of the tested materials on cowpea seeds at storage periods (3 months) was represented in Table 3. The result showed that $2LC_{95}$ of fenugreek, ginseng and marjoram oil have a reduction effect on F_1; which decreased gradually from 100% at initial time till reached 21.26, 51.97 and 20.46%, respectively at the 6th week. On the other hand, LC_{95} of abamectin decrease the number of F_1 to zero (100% reduction) in all times of treatment until storage periods (3 months).

The percentage of *C. maculatus* emergence in cowpea seed treated with the tested mixtures) showed that mixture 1 (2 LC_{95} fenugreek - LC_{20} abamectin) have reduction effect on the progeny of *C. maculatus*. This reduction recorded 100% at the initial time, and slowly decreases till reached 85.22% at the 12th week. On the other hand, mixture 2 (2 LC_{95} ginseng oil - LC_{20} abamectin) showed high reduction effect on emerged *C. maculatus* which recorded 100% at the initial time up to 12th week without any decrease in the percentage all over the period of storage. For mixture 3 ($2LC_{95}$ marjoram - LC_{20} abamectin) the results indicate that, this mixture has a high reduction effect on the emergence of *C. maculatus* and the reduction percentage recorded 100% at the initial time then show a significant stability till the 8th week and begin decreases to 97.85% at the 10th week till reaches 94.75% by the end of storage period (at 12th week).

Effect of the tested materials and mixtures on cowpea seeds germination

Data represented in Fig. 1 showed that germination of cowpea seeds treated with both marjoram oil and abamectin insecticide remained almost equal to the control (100%) at the initial and after storage period (6 weeks). Fenugreek and ginseng indicate 2% reduction in germination at initial time only. Slight reduction in germination was observed with the tested mixtures at the initial and after storage periods.

TABLE 3. Percentage of C. maculatusprogeny (F_1) in cowpea seeds treated with the tested materials at storage periods.

Treatment	Reduction % of *C. maculatus* emergence at the storage periods (weeks)						
	Initial	2nd week	4th week	6th week	8th week	10th week	12th week
Fenugreek $2LC_{95}$	100	89.07	54.37	21.26	---	---	-----
Ginseng $2LC_{95}$	100	99.09	92.65	51.97	----	----	----
Marjoram $2LC_{95}$	100	70.85	54.99	20.46	----	---	----
Abamectin LC_{95}	100	100	100	100	100	100	100 till 3 months
Mix 1 (2 LC_{95} fenugreek - LC_{20}abamectin)	100	100	97.52	96.24	91.88	91.43	85.22
Mix 2 (2 LC_{95} ginseng oil - LC_{20}abamectin)	100	100	100	100	100	100	100
Mix 3 ($2LC_{95}$ marjoram - LC_{20}abamectin)	100	100	100	100	100	97.58	94.57

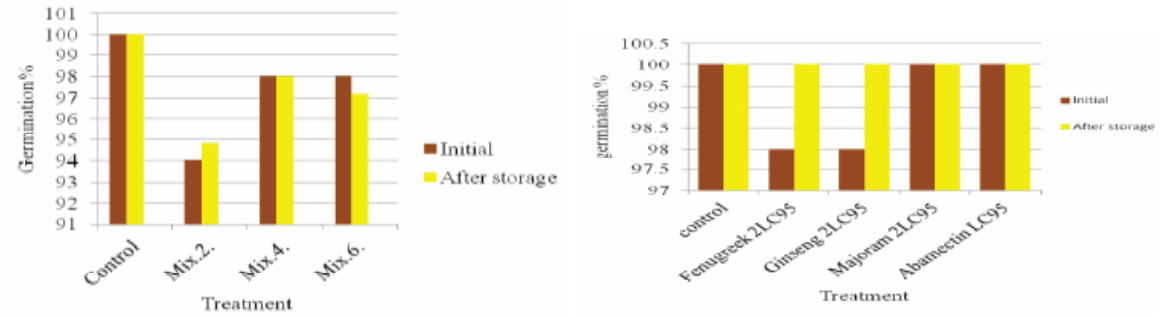


Fig. 1. Germination percentage of cowpea seeds treated with the tested materials and their mixtures at initial treatment and after storage period.

Cytological studies

Effect of the tested materials on root tip cells of Vicia faba plant

The results obtained showed that treatment for 24 h induced reduction in mitotic index (MI) in all roots treated with different concentrations of the tested materials as compared with the negative control (H_2O); while there is an increase in the mitotic index values as compared with the positive control (petroleum ether). Reduction in MI increased with the increase of the concentration applied (Table 4 and Fig. 2). It recorded 2.42, 2.01 and 1.64% in roots treated with the highest concentration of fenugreek, ginseng and marjoram respectively, as compared with negative control (4.18%) or with the positive control (1.83%). This result indicates that petroleum ether has more inhibition effect on mitotic division than oils. Despite such reduction in MI values, there is no complete inhibition of any mitotic stage.

MI value decreased gradually with the increase of abamectin concentrations. It reached the lowest value of 0.91% in roots treated with the highest concentration (LC_{95}) of abamectin as compared with the negative or the positive control. On the other hand, all the mixtures applied recorded significant decrease in MI values. Mixture 3 recorded the lowest value (1.50%); while mixture 1 and mixture 2 recorded 1.87 and 1.55%, respectively. Mixture 1 showed slightly increase in MI (1.87%) as compared with LC_{20} abamectin which recorded 1.60% (Table 4).

From Table 5 and Fig. 3, it can be noticed that the tested oils and abamectin induces different types of mitotic abnormalities in different mitotic stages and their frequencies increased gradually with increasing the concentrations applied. The mean percentage of total abnormal mitosis recorded 42.42, 43.20, 37.68 and 63.46% in roots treated with the highest concentration of fenugreek, ginseng, marjoram and abamectin respectively as compared with the negative control which recorded 0.63%. The frequency of abnormal mitotic phases in roots treated with mixtures 1, 2 and 3 recorded 42.22, 48.14 and 53.68%, respectively. The statistical analysis of data reveals that, the highest concentration ($2LC_{95}$) of oils had significant effect while, the lowest concentrations (LC_{50}) had no significant effect. On the other hand, all the concentrations of abamectin had a significant effect.

The most conspicuous effect of the tested materials was the complete inhibition of the spindle fibers formation leading to the appearance of C-metaphase (Table 5). The most common abnormalities at anaphase-telophase stages were the formation of bridges and disturbed chromosome. Breaks appeared with low percentage, while high percentage of sticky phases was appeared especially at the high concentration used (Fig. 3).

Discussion

Toxicological effects of the tested materials on Cowpea weevil (Callosobruchus maculatus)

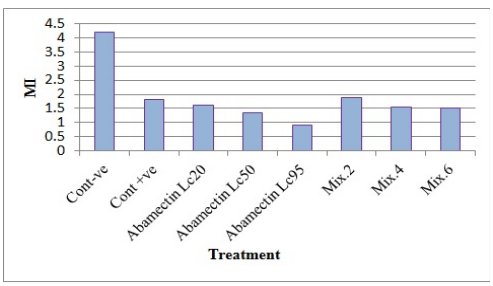
Results obtained from this study revealed gradual increase in the mortality percentage of the *C. maculatus* adults with increasing concentrations of the tested plant oils and abamectin. Similar findings are obtained by some investigators. Koumaglo et al. (1998) declared that essential oil of *Cymbopogon schoenanthus* had a toxic effect on the various developmental stages as well as on the adults of *C. maculatus*. Maina & Lale (2004) showed that neem oil produced 100% mortality in the various developmental stages of *C. maculatus*; using petroleum ether extract of this oil. Ileke & Olotuah (2012) declared that oil extracts of *Anacardium occidentale* (L.) seeds and *Allium sativum* (L.) bulbs

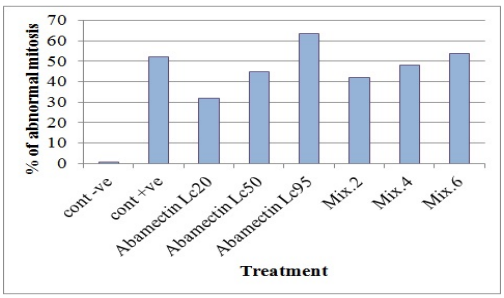
were effective in controlling cowpea bruchid, C. maculates in stored cowpea seeds. Habou et al. (2014) clarify the insecticidal efficacy of *Jatropha curcas* seed oil against two beetle species, *Callosobruchus maculates* Fab and *Bruchidius atrolineatus*.

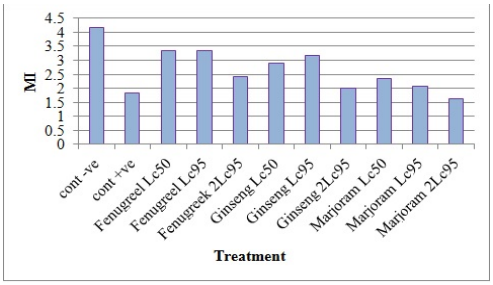
TABLE 4. Total cell examined, total mitosis, total abnormal mitosis, percentage of abnormal mitosis and mitotic index after treating Vicia faba root tips with different concentrations of the tested materialsfor 24 h.

Treatment		Total cell examined	Total mitosis	Mean % of Mitotic Index ± SE	Total abnormal mitosis	Mean % of abnormal mitosis ± SE
	LC_{50}	7430	250	3.36 ± 0.77	63	25.20 ±0.15
Fenugreek oil	LC_{95}	6520	219	3.36 ± 0.20	79	36.07 ±0.12
	$2LC_{95}$	6815	165	2.42 ± 0.14*	70	42.42 ±0.16*
	LC_{50}	7325	212	2.89 ± 0.16	57	26.88 ±0.19
Ginseng oil	LC_{95}	6514	208	3.19± 0.49*	67	32.21±0.04*
	$2LC_{95}$	6212	125	2.01± 0.05*	54	43.20 ±0.28*
	LC_{50}	7005	165	2.36± 0.35	31	18.79 ±0.19
Marjoram oil	LC_{95}	7618	158	2.07± 0.08*	43	27.22 ±0.22
	$2LC_{95}$	4210	69	1.64± 0.30*	26	37.68±0.16*
	LC_{20}	6895	110	1.60 ± 0.06*	35	31.82 ±0.18*
Abamectin	LC_{50}	6350	85	1.34 ± 0.12*	38	44.71 ±*
	LC_{95}	7225	66	0.91 ± 0.09*	42	63.46 ±0.23*
Mixture 1 (LC20 abamectin + 2LC95 fenugreek oil)		7210	135	1.87 ± 0.34*	57	42.22 ±0.29*
Mixture 2 (LC20 abamectin + 2LC95 ginseng)		6950	108	1.55 ± 0.16*	52	48.14 ±0.05*
Mixture 3 (LC20 abamectin + 2LC95 marjoram)		6320	95	1.50 ± 0.09*	51	53.68 ±0.16*
Negative control (H2O)		7650	320	4.18 ± 0.05	2	0.63 ±0.15
Positive control (Petroleum		6820	125	1.83 ± 1.06	65	52.00 ±0.14

*= Significant from the control at the 0.05 level. ($P \leq 0.05$).







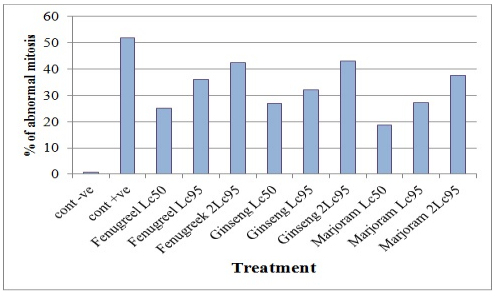


Fig. 2. Percentage of mitotic index and total abnormal mitotic phases after treating *Vicia faba* root tips with different concentrations of the tested oils, abamectin and their mixtures.

TABLE 5. Percentage of different types of mitotic abnormalities after treating *Vicia faba* root tips with different concentrations of the tested oils, abamectin and their mixtures for 24 h.

Treatments		Abnormal Prophase %	% of different types of metaphase abnormalities						% of different types of anaphase-telophase abnormalities						
			CM	Break	Stick	Disturbed	Laggard.	Total	Laggard	Bridge	Multi	Break	Stick	Disturbed	Total
Fenugreek oil	LC_{50}	6.41	11.90	------	18.23	5.59	2.38	38.10	-----	5.68	1.14	-----	17.05	5.68	29.55
	LC_{95}	15.15	11.76	2.53	22.36	5.88	3.53	46.06	-----	7.35	4.41	4.41	22.06	4.41	42.64
	$2LC_{95}$	13.04	6.58	1.32	44.74	------	3.95	56.58	-----	6.98	2.33	-----	32.56	2.33	44.19
Ginseng oil	LC_{50}	15.09	4.40	2.20	21.98	------	3.30	31.87	-----	7.35	1.47	8.82	11.76	-----	29.4
	LC_{95}	23.53	4.22	7.37	21.05	------	1.05	33.69	-----	8.06	3.22	8.06	15.15	1.61	36.10
	$2LC_{95}$	24.24	15.87	4.76	31.75	------	3.17	55.56	-----	3.45	3.45	-----	27.59	3.45	37.93
Marjoram oil	LC_{50}	17.24	3.23	9.68	3.23	1.61	1.61	19.35	-----	4.44	2.22	2.22	6.68	4.44	20.00
	LC_{95}	24.49	3.33	5.00	8.33	3.33	1.67	21.67	-----	6.33	1.27	2.53	26.60	-----	36.73
	$2LC_{95}$	30.00	5.77	------	22.22	------	2.78	30.77	-----	8.70	-----	8.70	34.78	-----	52.17
Abamectin	LC_{20}	21.88	11.54	------	11.54	7.69	7.69	38.46	3.85	9.62	1.92	-----	13.46	5.77	34.62
	LC_{50}	36.36	8.33	------	12.51	12.51	8.33	41.68	5.12	14.82	-----	-----	23.38	7.96	51.28
	LC_{95}	61.54	7.41	------	51.85	7.41	------	66.67	-----	18.52	3.70	-----	39.32	-----	61.54
Mixture 1		13.16	19.32	4.67	23.81	4.67	4.67	57.56	-----	14.54	-----	3.64	27.27	5.45	50.90
Mixture 2		9.38	11.11	4.44	33.35	4.44	2.22	55.56	3.23	27.96	1.62	6.45	31.71	6.45	77.42
Mixture 3		12.00	9.38	15.63	56.25	------	------	81.25	-----	15.78	-----	2.63	34.22	5.26	57.89
Control	negative	0.00	------	------	------	2.11	------	2.11	-----	-----	-----	-----	-----	-----	0.00
	positive	26.32	8.70	2.17	54.35	10.87	------	76.08	-----	9.79	-----	-----	29.20	9.79	48.78

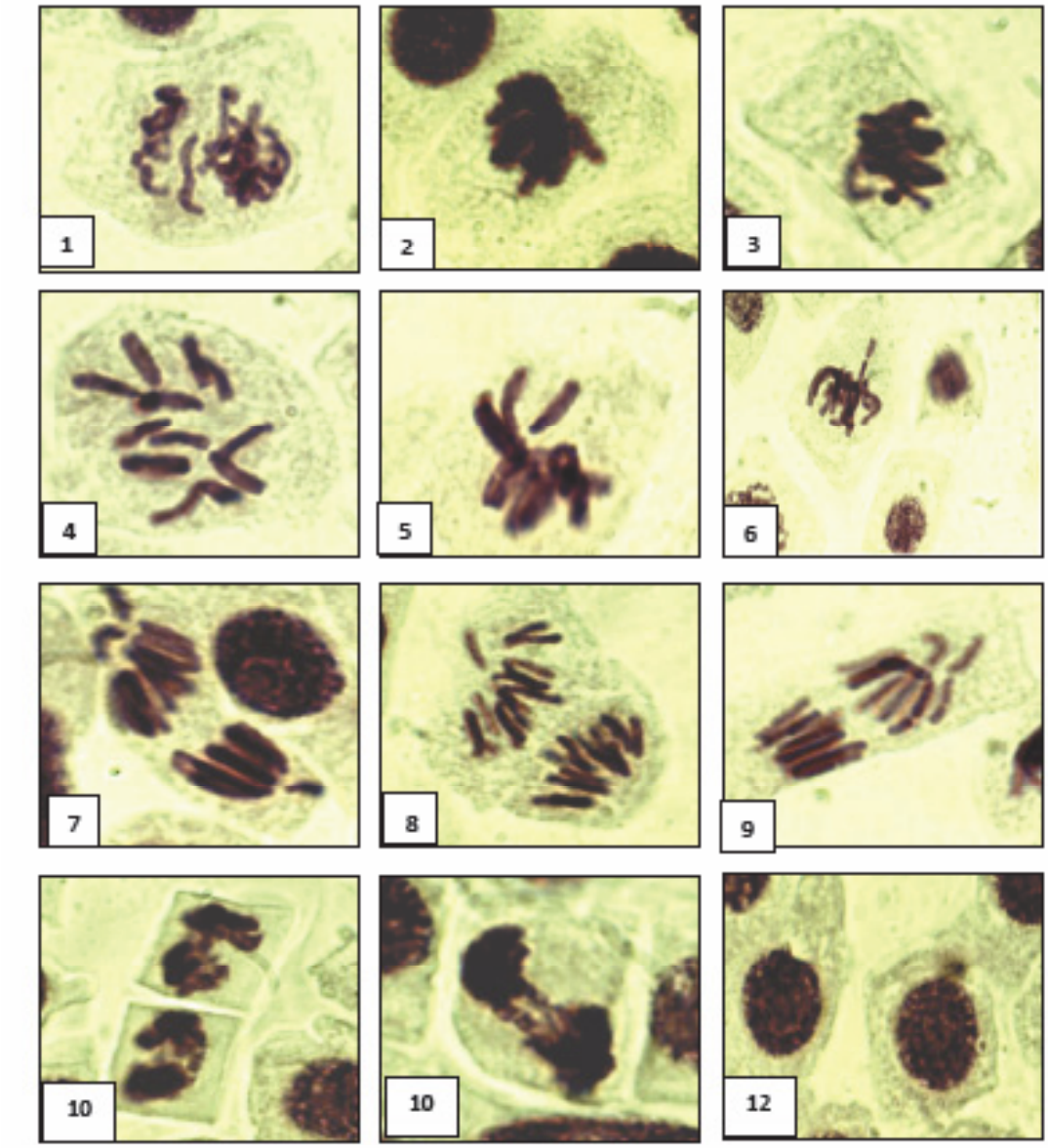


Fig. 3. Different types of abnormal mitotic phases after treating *Vicia faba* roots with different concentrations of the tested oils, abamectin and their mixtures (1 = Abnormal prophase; 2, 3 = Sticky metaphase; 4 = C-metaphase; 5 = Lagging chromosome at metaphase; 6 = Fragment at metaphase; 7, 8, 9 = Lagging chromosome at anaphase; 10, 11 = Bridge at telophase; 12 = Micronucleus at interphase).

The difference observed among the insect mortalities treated with plant oils was due to differences in their volatiles. Missed found that monoterpenes present in most essential oils were very active on insect due to their active volatiles. The essential oils of plant origin are highly lipophilic and subsequently have the ability to penetrate the cuticle of insects. By this process, the plant material, may act as a contact poison which may exert a toxic effect by disrupting normal respiratory activity of the weevils (Richards, 1978). Thereby resulting in asphyxiation and subsequent death (Umar, 2013). On the other hand, Anuradha et al. (2007) reported that in Drosophila melanogaster cells, azadirachtin isolated from neem plant induced depolymerization of actin, leading to cell cycle arrest and subsequently apoptosis.

Residual activity of the tested materials on the cowpea seeds showed that LC95 of abamectin gave a complete protection to treated cowpea seeds against *C. maculatus* infestation (100% reduction) in all times of treatment until storage periods (3 months). Abamectin mode of action is associated with its effect on the aminobutyric acid receptors and glutamate-gated chloride channels increasing the permeability of chloride ions disturbing the neuromuscular transmission leading to deathmissed . On the other hand, in the current study, 2LC95 of fenugreek, ginseng and marjoram oil have a reduction effect on F1; which decreased gradually from 100% at initial time till reached 21.26, 51.97 and 20.46%, respectively at the 6th week. This result is agreement with Habou et al.(2014) who reported that the *Jatropha curcas* oil was toxic to *C. maculatus* after 7 days and the rates of emergence of the insect was 76.2%, indicating that exposure to *Jatropha* oil drastically reduced adult emergence.

The three mixtures used in this study, showed high reduction effect on emerged *C. maculatus*. The most effective is mixture 2; the reduction percentage recorded 100% at the initial time up to 12th week without any decrease in the percentage all over the period of storage. Mixture 1 and 3 recorded 100% at the initial time till reached 85.22% and 94.75% respectively, at the12th week. Mujica et al. (2000) evaluated the validation of abamectin applied alone or mixed with plant oil on Leaf miner fly (LMF), *Liriomyza huidobrensis*, on bean plants, and declared that plant oils increased the efficacy of abamectin to the extent that the active ingredient of this bio-insecticide could be reduced by one-half to three-fourths of the common dosage.

Cowpea seeds germination was not affected by tested oils or by abamectin application at the initial time and at the end of the storage period (3 months). These data indicated that, oils or the bio-insecticide abamectin at this dose did not harm on seed viability. In consistence with these results, Abdel-Salam (2005) found that, the garlic oil, ethyl oleate, sesame oil and flax oil have no adverse effect on seed germination when applied on cowpea seeds till the end of the storage period. On the other hand, Mohamed & El-Ashry (2012) cleared that the percentage of germination reached to less half in Pisum sativum stored for three months and treated with fenugreek extracts (2%) as compared to control, but percentage of germination improved after storage for 6 months. They reported that the fenugreek extracts with low concentration in storage the seeds are used safely.

Cytological studies

Plant bioassays such as that measure mitotic division and the frequency of chromosomal aberrations are efficient and simple in genotoxicity studies. MI could be considered as a delay in the cell proliferation kinetics and may be considered a reliable test for monitoring toxicity levels, whereas chromosomal aberration was reported to be a good indicator to access the mutagenicity of chemical *in vivo* (Rojas et al., 1993 and Grant & Owens, 2006). One of the major effects of the tested materials was shown on their influence on cell division activity. A drastic reduction in mitotic indices was clearly observed in all treatment with the tested materials as compared with negative control. It can be concluded that the bio-insecticide abamectin is the more effective in inducing reduction in mitotic activity.

The reduction in mitotic activity was referred to blocking of mitotic cell cycle preventing the cell from entering mitosis, inhibition of DNA or protein synthesis or induction of large numbers of mitotic abnormalities (Binarova et al., 1998). Arresting cells at G1 or G2 periods of cell cycle have been registered by a number of studies, as a consequence of cyclin-dependent kinases (CDKs) synthesis inhibition (Mitra et al., 2012 and Nejad et al., 2012). Mohamed & El-Ashry (2012) showed that the cytophotometric measurements for *Pisum sativum* root cells treated with Fenugreek extracts and stored three months indicated the accumulation of cells at G_1 phase at the expense of other phases of the cell cycle; in addition to the lower values of mitotic index.

The oils and the biocide abamectin examined in this work induced number of chromosomal aberrations in all mitotic phases. Disturbances in the mitotic division, like spindle inactivation, causes C-mitosis, disturbed metaphase and other irregularities in the chromosome distribution during anaphase. Laggards chromosome was also recorded which may reflect the failure of chromosomes to attach to the spindle fibers. This stress in chromosome movement may result in fragmentation of chromosomes (Kavitha, 2008 and Khanna & Sharma, 2013).

Chromosomal stickiness was also observed in

different mitotic stages in roots treated with high concentration of oils and abamectin. Babich et al. (1997) reported that metaphases with sticky chromosomes forfeit their normal appearance and appear to have a sticky "surface" which causes chromosome agglomeration, possibly due to effects on chromosome organization. Chromosome bridges observed in this study are mostly due to stickiness and this quit apparent in root treated with high concentration. Micronuclei in interphase cells were observed after treatment with abamectin. The creation of micronucleus has been used in many studies as an indicator of genotoxicity (Cavas & Ergene-Gozukara, 2005). These abnormalities illustrate reflections of structural and/or numerical chromosomal aberrations arising during mitosis (Kada et al., 1985 and Khanna & Sharma, 2013).

Results obtained from percentage of total abnormalities showed that there is no change in frequency of abnormalities between $2LC_{95}$ fenugreek oil and their mixture with the lowest concentration of abamectin (LC_{20}), while there is slightly increase in aberration frequency in roots treated with $2LC_{95}$ Ginseng as compared with mixture 2 (LC_{20} abamectin- $2LC_{95}$ ginseng). In consistence with the protective effect of fenugreek, Kaviarasan et al. (2004) evaluated polyphenol-rich extract from the seeds of fenugreek against hydrogen peroxide (H_2O_2) in human erythrocytes (RBCs) and showed that fenugreek seed extract significantly reduced the oxidative modifications. These findings elucidated the potent antioxidant properties of the fenugreek seeds. On the other hand, the results obtained in this study that related with *Origanum majorana* and their mixture with abamectin are not in agreement with that of Qari (2008) who showed that *Origanum majorana* extracts significantly reduced the total number of aberrations which were stimulated by sodium azide. Also, Khatab & Elhaddad (2015) demonstrated that the 1.25µl of *Origanium majorana* oils have the potency to suppress mono-sodium glutamate effect by increasing of mitotic index and reduction of the chromosomal aberration and thus could be a promising antimutagenic and antigenotoxic potential.

Of the results obtained, the plant oils alone do not offer total exclusion of cowpea weevil and the synergistic impact shown by the mixture of abamectin and plant oil permits a reduction in the commercially recommended concentration of abamectin without any loss in effectiveness. Thus, treatment costs can be reduced and farmers will be able to use abamectin to control storage pests.

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(Received 12/11/2017; *accepted* 29 / 1 /2018)

افاق المبيدات الحيوية فى المستقبل فى مكافحة الافات

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استهدفت هذه الدراسة التعرف على مواد طبيعية ذات كفاءة عالية فى مكافحة حشرة خنفساء االلوبيا (*Callosobruchus maculatus*) - والتي تعتبر من أخطر الحشرات التى تسبب ضرراً كبيراً للبذور المخزنة خاصة بذور العائلة البقولية - تكون بديلاً عن المبيدات الكيميائية و أكثر أماناً للإنسان و البيئة المحيطة. ولذلك فقد تم في هذا البحث تقييم سمية ثلاثة أنواع من الزيوت المستخلصة من نباتات الحلبة والجنسنج والبردقوش بالإضافة إلى المبيد الحيوي أبامكتين(Abamectin) المستخلص من *Streptomyces avermitilis* على هذه الحشرة وكذلك على كل من نباتي الفول واللوبيا. أوضحت النتائج وجود علاقة طردية بين النسبة المئوية لموت الحشرات والتركيز المستعمل وكذلك مع مدة المعاملة، حيث سجل المبيد أبامكتين فاعلية عالية تجاه الطور البالغ للحشرة البالغة وأحدث التركيز LC95 إبادة كاملة لها بنسبة 100% حتى إنتهاء الفترة المحددة لتخزين بذور اللوبيا (ثلاثة أشهر)، بينما لم تتعد قدرة الزيوت الثلاثة على تسجيل نسب موت للطور البالغ (100%) سوى بضعة أيام، تبعها إنخفاض حاد في كفاءة الزيوت. كما استطاع المبيد أبامكتين (LC_{95}) منفرداً أوالخليط المكون من المبيد والجنسنج (LC_{20} abamectin-2LC_{95} ginseng) منع ظهور حشرات من الجيل الأول بنسبة 100% حتى إنتهاء فترة التخزين، يليه خليط المبيد والبردقوش (LC_{20} abamectin-2LC_{95} marjoram) والذي كان فاعلاً بنسبة 94.57%، ثم الخليط (LC_{20} abamectin - 2LC_{95} fenugreek) والذي سجلت فاعليتة نسبة 85.22%. من ناحية أخرى تم دراسة التأثير السيتولوجي للمواد السابقة على جذور نبات الفول وأوضحت النتائج أن جميع المعاملات لمدة 24 ساعة أدت إلى حدوث تغير في نسب الأطوار الميتوزية المختلفة بالإضافة إلى حدوث إنخفاض ملحوظ في معدل الإنقسام وأن هذا الإنخفاض يزداد بزيادة التركيز، وأن المبيد الحيوي كان أكثرهم تأثيراً يليه زيت البردقوش، الجنسنج ثم الحلبة. أدت المعاملات أيضاً إلى ظهور عدداً من الشذوذات الكروموسومية، حيث كانت نسبتها في الجذور المعاملة بالزيوت أقل كثيراً من مثيلتها في الجذور المعاملة بالمبيد منفرداً أو بعد خلطه مع الزيوت. من أهم هذه الشذوذات اللزوجة، الطور الإستوائي الكولشيسيني، التشتت الكروموسومي في الطور الإستوائي أو الإنفصالي، القناطر الصبغية، الكروموسومات الحائرة، الخلايا ذات الأنوية الدقيقة بالإضافة إلى ظهور عدد قليل نسبياً من الكسور الكروموسومية.