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QUARANTINE TREATMENT ON CUT FLOWERS BY NATURAL FUMIGANTS

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ABSTRACT

Quarantine pest infestation is a serious limiting factor for export of cut flowers from Israel. Currently, the measures to control these insects rely mainly upon the use of the fumigant methyl bromide (MB), which has been classified as a compound with ozone-depleting potential. The ultimate ban of this fumigant under the conditions of the Montreal protocol will have serious impacts on the post harvest commodity treatments, unless suitable alternatives are developed. This study is aimed to search for suitable ecologically safe alternatives to this fumigant for quarantine and other purposes.

Aromatic plants are known to synthesize chemicals of secondary metabolic importance which when isolated, are toxic or repellent to many insects but harmless to mammals. Their volatility and insecticidal efficiency make them good prospective alternative fumigants to MB. By screening a large number of essential oils and their active components, extracted from aromatic plants, using fumigation chambers, we could be able to isolate a number of very promising compounds for the control of insects, including the common quarantine pests attacking cut flowers for export. Against the white fly *Bemisia tabaci* and thrips *Frankliniella occidentallis* a low concentrations of 10 and 20 g/m^3 and exposure times of 2 and 4 hours respectively were needed to obtain 100% mortality. No phytotoxicity was observed 7 days after fumigation. For the control of the leaf miner *Liriomyza trifolii* and snails *Xeropicta vestalis joppensis* a higher concentrations of 50-60 g/m^3 are needed. The phytotoxicity of these compounds is dependent on the species and the varieties of the flowers, also on the temperature during fumigation and the flowers storage conditions, especially cooling before and after treatment.

INTRODUCTION

Cut flowers are a major agricultural product for export from Israel. Annually about 1.5 billion flowers, at a value of US$ 200 million are exported from Israel. Nevertheless, export of cut flowers from Israel is subject to regulations of importing countries regarding infestation by several quarantine pests. Currently, the most effective method to control these pests at the post harvest stage is by fumigation with methyl bromide (MB). However, in the near future, cut flowers treated by this compound will be not acceptable on the world markets, especially the European, because of strong environmental demands of certain international organizations such as MPS with regard to the negative influence of MB on the ozone layer of the atmosphere. MB has been identified as a contributor to ozone depletion (Anon. 1995), and will be phased out in 2005 in developed countries and in 2015 in developing countries.

Therefore, there is an urgent need to search for suitable ecologically safe alternatives to this fumigant for quarantine and other purposes.

Throughout the centuries, traditional agricultural practice in developing countries has developed an effective means for insect pest control using materials of botanical origin. However, their efficacy and optimal use needs to be assessed in order to render these cheap and simple means of insect control available for public use. Aromatic plants contain volatile compounds that are known to possess insecticidal and insect-repellent activities. These allelochemical compounds are mainly essential oils (Brattsten, 1983; Schmidt *et al.,* 1991; Shaaya *et al.,* 1991, 1998). Their high volatility makes it possible to extract the oils by water or steam distillation. Most of the essential oil constituents are monoterpenoids, which are secondary plant chemicals and considered to be of little metabolic importance. One of the first pieces of evidence to be realised on the biological activity of essential oils was that of the resistance of the Scotch pine to flat bugs, which was related to the high content of essential oils in the bark (Smelyanets and Khursin, 1973).

The mechanism of the toxic effect of essential oils is not yet clear. Ryan and Byrne (1988) reported that linalool inhibits the enzyme acetylcholinesterase (AChE). In our studies (Greenberg *et al.,* 1993), we were able to show that a number of monoterpenes are competitive inhibitors for AChE of some stored product pests such as *Rhyzopertha dominica* and *Tribolium confusum*. A correlation between the relative toxicity of the oils and the relative inhibition of the enzyme activity in the tested insects was established. The failure of the biologically active terpenes to produce strong enzyme inhibition *in vivo* and *in vitro*, leads us to postulate that the enzyme AChE is not the main site of action of the monoterpenes (Greenberg *et al*., 1993). Neurotoxicity of several terpenes was also studied (Coats *et al.,* 1991). We are currently evaluating some other hypotheses, for example, the influence on the octopamine system.

In our previous studies the fumigant toxicity of around 50 essential oils extracted from various aromatic plants and some of their major constituents were assessed against various developmental stages of some stored-product insects. We were able to show that the biological activity of these oils is insect stage, and species specific. Several compounds were found to be active fumigants at low concentrations against these insects (Shaaya *et al.,* 1991, 1993, 1994, 1997; Shaaya and Kostjukovsky, 1997, 1998).

This study is aimed at evaluating the potential of natural volatiles, mainly essential oils, as possible MB substitutes for quarantine pest control on cut flowers. Most of these compounds are generally regarded as safe (GRAS). A large number of essential oils, extracted from aromatic plants, were screened against the main quarantine insects of cut flowers in Israel: western flower thrips *Frankliniella occidentalis* Gennadius (Thysanoptera: Thripidae), tobacco white fly *Bemisia tabaci* Pergande (Homoptera: Aleyrodidae), leaf miner *Liriomyza huidobrensis* L. (Diptera: Agromyzidae) and snails *Xeropicta vestalis joppensis* Schmidt. The phytotoxicities of these treatments to rose, dianthus and gypsophila were also evaluated.

TABLE 1
Fumigant activity of a number of essential oils on the quarantine pests of cut flowers

Compound	Whitefly *Bemisia tabaci*			Thrips *Frankliniella occidentalis*			Leaf miner *Liriomyza huidobrensis*		
	Conc. g/m^3	Exposure time (h)	Mortality %	Conc. g/m^3	Exposure time (h)	Mortality %	Conc. g/m^3	Exposure time (h)	Mortality %
O89	10	2	100	20	4	67			
C31	15	2	100	20	4	100*	50	2	89
C72	10	2	100	20	4	94			
C33	20	4	100	20	4	67	50	2	97
O3				20	4	100			
ZP51							50	2	100
MB	15	2	100	20	4	100**	30	3	100

No phytotoxicity was recorded 7 days after treatment except low phytotoxicity denoted by **.

Temperature during treatment was 25ºC. Mortality was recorded: 3 h after treatment for whitefly, 24 h – for thrips, 48 h – for leaf miner.

MATERIALS AND METHODS

The tested insects were laboratory strains of *B. tabaci* (whitefly), *F. occidentalis* (thrips) and *L. huidobrensis* (leaf miner), which were reared on cotton or bean plants. The snails *X. vestalis joppensis* were collected in the field. For fumigation activity tests, 1.5-L glass chambers closed hermetically were used. The tested whitefly adults were placed in specially designed cages, perforated with small holes to allow even distribution of gases. Thrips adults were introduced into the rose flower. Leaf miner larvae developed inside gypsophila leaves that were exposed previously to leaf miner adults. Snails were placed in the chambers on the tested plants. The essential oils or their constituents were applied to a small piece of filter paper (Whatman, No. 1) and suspended in the fumigation chamber. To obtain even distribution of the gases during treatment, a magnetic stirrer was used for the duration of the treatment. Twenty adult thrips and white fly or leaf miner larvae were used for each replicate.

Editor's note: the composition and names of these compounds are retained by the authors.

Temperature during fumigation was 25°C, and during the phytotoxicity test, 5 and 25°C. Mortality counts were performed 3 h after treatment for whitefly, after 24 hours for thrips and after 48 h for leaf miner. The data are presented after Abbott's correction. Phytotoxicity was tested under controlled temperature-humidity conditions (20ºC, 60% r.h.) 7 days after treatment and scored at levels of: 0 - no phytotoxicity; 1 - low, 2 - medium, and 3 - high phytotoxicity.

TABLE 2
Phytotoxicity of plant volatiles on dianthus (var. "Rendezvous") and gypsophila

Compound	Conc. (µL/L)	Exposure time (h)	Phytotoxicity	
			Dianthus	Gypsophila
C-72	30	4	0	0
O-89	30	4	1	0
O-33	30	4	0	0
O-35	30	4	0	0
C-31	15	2	3	0.1
	30	4	3	2
O-88	15	2	3	0.1
	30	4	3	3
O-75	30	4	2	1

1.5-L fumigation chambers were used. Temperature during fumigation and phytotoxicity tests was 25ºC. Phytotoxicity was tested 7 days after treatment.

RESULTS

By screening a large number of essential oils and their active components, extracted from aromatic plants, using fumigation chambers, we were able to isolate a number of very promising compounds for the control of the common quarantine pests attacking cut flowers. The toxic effect of the essential oils on various insects was different. Against the whitefly *B. tabaci* and thrips *F. occidentalis* a low concentrations of 10 and 20 g/m^3 and exposure time of 2 and 4 h respectively were needed to obtain 100% mortality (Table 1). No phytotoxicity was observed 7 days after fumigation. For the control of the leaf miner *L. huidobrensis* and snails *X. vestalis joppensis* a higher concentrations of 50-60 g/m^3 were needed. Some compounds (C72, O89) were found to be very active on whitefly *B. tabaci* and thrips *F. occidentalis,* but showed a much less toxic effect on the leaf miner *L. huidobrensis.* It was shown that the insecticidal activity of these compounds depends not only on their concentration and exposure time, but also on the temperature during fumigation. In fumigation by the essential oil constituent C72, thrips mortality at 30°C was 94% compared with only 30% at 20°C (data not shown).

Phytotoxicity of these compounds depends on the species and variety of the flowers, on the temperature during fumigation, and on the storage conditions of the flowers, especially on cooling before and after treatment. Thus, carnations were much more sensitive to the treatment by essential oils compare with gypsophila (Table 2). The carnation variety "Duna" was more tolerant to compounds O33 and O35 compared with variety "Albin" (Table 3). Keeping the treated flowers at a low temperature prevented the phytotoxic effect. When the carnation variety "Rendezvous" was kept after fumigation at 5°C no phytotoxicity was recorded, whereas at 25°C some oils and their constituents revealed a phytotoxic effect (Table 4).

TABLE 3

Phytotoxicity of two plant volatiles O-33 and O-35 on various varieties of dianthus

Variety	Conc. (μL/L)	Exposure time (h)	Phytotoxicity	
			O-33	O-35
Albin	20	3	1.0	1.0
Delphi	20	3	0.1	0.1
Duna	20	3	0	0
Guri	20	3	0.1	0.1
Kennedy	20	3	0.1	0.1
Rimini	20	3	0.1	0.1
Sheri	20	3	0.1	0.1

1.5-L fumigation chambers were used. Temperature during fumigation was 25ºC, and 5ºC during phytotoxicity tests. Phytotoxicity was tested 7 days after treatment.

TABLE 4

Effect of storage temperature on phytotoxicity of plant volatiles on dianthus (var. "Rendezvous")

Compound	Conc. (μL/L)	Exposure time (h)	Phytotoxicity	
			5ºC	25ºC
C-72	15	2	0	0.1
O-33	15	2	0	0
O-35	15	2	0	0
O-89	15	2	0	0.1
C-31	15	2	0	3

1.5-L fumigation chambers were used. Temperature during fumigation was 25ºC. Phytotoxicity was tested 7 days after treatment.

CONCLUSIONS

Aromatic plants are known to synthesize chemicals of secondary metabolic importance, mainly essential oils, which when isolated, are toxic to many insects but harmless to mammals and friendly to the environment. Their volatility and insecticidal efficiency make them good prospective alternative fumigants to MB for control of quarantine pests in cut flowers as well as control of other insect pests. We also expect that this study will open new avenues for the use of essential oils in other fields of agriculture and veterinary medicine.

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