World Applied Sciences Journal 28 (12): 1913-1917, 2013
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2013.28.12.2209

Investigation on the Insecticidal Efficacy of Novel Pellet Formulation Against *Challosobruchus maculatus* f. (Col.: Bruchidae) in Three Different Heights and Compared with Phosphin

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Abstract: Applying essential oils fumigant toxicity in stored products pests control has sharpened recently. Since these products have low penetration power and vapor pressure, their use in crop depth is restricted. The aim of this work was using controlled release technology to solve this problem. In this research, 1,8-Cineole based pellets were prepared by Dry Mixing Method and physical load of 1,8-Cineole on strach, which followed by pressing the mixture, using pellet maker apparatus, to form pellets. Insecticidal efficacy of produced pellets was investigated against adults (1-3 days old)*Challosobruchusmaculatus* F. (Col.: Bruchidae)compare with phostoxinunder 28±2°C and darkness in laboratory condition.$LC_{50}$and$LC_{95}$of pellets against *C.maculatus*in 24h0.017 and 0.050 ml active ingredient per L air, respectively.LC_{50}andLC_{95}ofPhosphin pellets against *C.maculatus*in 24h 0.124 and 1.852 ml active ingredient per L air, respectively.

Key words: Controlled release · 1 · 8-Cineole · Acetone · Starch · Pellet formulation · Phosphin · Fumigant toxicity

INTRODUCTION

Fumigation plays the major role in insect pest elimination in stored products. Pesticides fumigation played a major role in destroying insect pests and storage of pesticides and the pesticide methyl bromide and phostoxin are the most used [1]. Consumption of methyl bromide and phostoxin fumigation and pesticides had been reduced and these compounds are now [2]. Insect resistance to common fumigants such as phosphine etc. is a global issue now and control failures have been reported in field situations in some countries [1-4]. In addition, there have been some arguments about the genotoxicity of phosphine [5]. Studies on fumigant toxicity of essential oils of plants and their constituents have been sharpened [6]. Eucalyptus essential oil is one of the most important essential oils which have fumigant toxicity against insects. 1, 8-Cineole is the most important compound of the genus Eucalyptus and is largely responsible for its pesticidial properties [7]. Controlled release technology has emerged as an alternative approach with the promise to solve the problems accompanying the use of some agrochemicals, while avoiding possible side effects with others [8]. The overall aim of controlled release formulation consists of protecting the supply of the reagent and allowing its automatic delivery to the target at controlled rates to maintain its concentration within an optimum concentration over a long period of time. Also controlled release technology could assist in improving protection for stored grains against insect and rodent pests [9]. Two different approaches have been reported in the case of the physical combination of biologically active agent with polymeric materials. Firstly the biologically active agent can be encapsulated in a polymeric material and insecond approach biologically active agent is heterogeneously dispersed or dissolved in a solid polymeric matrix which can be either biodegradable or non-biodegradable. Acetone as a solvent in many organic compounds used in the production of synthetic fibers, plastics and pharmaceuticals are used [10]. Using acetone no adverse effect on seed germination and germination rate index is not [11]. Acetonealso has the ability to publish Influenceinto the spaces filled [12].

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Acetonessuitablefor controllingvariouspestsare also toxic [11]. Starch is a natural polymer which possesses many unique properties and some shortcoming simultaneously. Some synthetic polymers are biodegradable and can be tailor-made easily.

Therefore, by combining the individual advantages of starch and synthetic polymers, Starch-based completely biodegradable polymers (SCBP) are potential for applications in biomedical and environmental fields. Therefore it received great attention and was extensively investigated [13].

MATERIAL AND METHODS

1, 8-Cineole with 154.25 g/mol molar mass and Assay (GC, area%) ≥ 98% was purchased from Merck (Darmstadt, Germany). Acetone (Molar Weight: 58.09 g/mol) was purchased from Merck (Darmstadt, Germany). Strach(Density: 1.5 g/cm^3) was purchased from Merck (Darmstadt, Germany).Phostoxin with 57.8 g/mol molar density 2.85 g/ cm^3 was purchased from C.MC CHAIN.

All culturing insects were obtained from laboratory cultures from Urmia University, stored product pests incubation room. Cowpea beetle, *C. maculatus* were reared on Cowpea at 12-13% r.h., respectively. All culturing media were purchased from a local market and kept in a freezer at -5°C for 48 h and then were used as culturing medium. Experimental procedures were carried out at 28±2°C in a dark room and 65±5% r.h. was provided only for culturing media. Experimental procedures were carried out at 28±2°C in a dark room and 65±5% r.h. was provided only for culturing media. Strach,Acetone and 1,8-Cineole mixture with various concentrations were prepared by Dry Mixing Method (DMM) in ice temperature on shacker with 145 rpm for 4 h. Preparation of mixture was taken place in tight 100 ml balloons and mixing power provided with shaker. Then prepared mixtures were transferred to FTIR pelletd esigned by the researcher and pellets were produced by pressure. Producing of a pellet from prepared mixture was taken about 1 min of manufacturing time. Average of ten 6 gr pellets diameter and thickness were measured by caliper that were 3±0.1 and 1±0.01 cm, respectively. Assessment of fumigant toxicity of pellets without grain was carried out with 20 adults (1-3 days old) exposed to 2200 ml barrel sealed with screwed metal caps fitted with nylon and adhesive tape. Volume of each barrel was measured by the amount of water it could contain. Preliminary trials in24h were undertaken on adults (1-3 days old), to determine optimum weight of pellets. Pellets were placed in barrel (90 cm in height and 56 cm in diameter) in each glass jar; hence, controls had no pellet in their barrel. Each container had perforated screen (40 meshes) at its end to enable penetration of volatiles emanating from pellet while insects had no contact with the pellet. 1, 2, 3 and 4 pellet with concentrations (1ml acetone and 1.2 ml 1, 8 Cineol active ingredients in 4.2 gr starch) was used as factorscompared 25, 50, 75, 100 and 125 gram of phostoxin pellet. According to preliminary trial results, one gram pellets were used asExperimental weight in original fumigant toxicity tests. Insect's mortality was determined immediately after treatment. Insects showing any movement by closing hot needle to their antenna were considered to be alive. Preliminary test for determination of optimum pellets weights caused mortality data, were altered in then were analyses by using the SPSS software. The dose-effect experiments were arranged by randomized complete design and the data were analyses with analysis of variance (ANOVA) by using the SPSS (V. 20) software. The LC50 and LC95 values were calculated by probit analysis (SPSS).

RESULTS AND DISCUSION

The fumigant activity of 1, 8-Cineole has been evaluated against similar insects [14-17]. Obtained results about susceptibility of experimental insects to 1, 8- Cineole, confirm prior researchers findings [15, 18]. In present study higher values of LC50 and LC95 were estimated for 1, 8-Cineole,, Acetone because a trace of 1, 8-Cineole and Acetone remained in polymer matrix in exposure time (Table 1, 2).

Preliminary test for determination of optimum pellets weight showed that insecticidal activity varied in all cases, with different pellet doses. Significant increase of mortality was observed in adults with increasing time and pellets. And increasing the height of the mortality rate has declined. According to preliminary trials, 4 pellets had significantly higher effect than other weights thus we used these pellets in our main experiments (Table 3-6).

Fumigant toxicity of pellets without grains against each of experimental species in 24h *C. maculatus*, is shown in Table 2. A significant increase of adult's mortality was observed in all cases with increasing doses. Fumigation containers volume were varied (e.g. 250, 500, 710 and 9000 ml) between different works [15-19]. Application of controlled release formulations in agriculture and study on different polymers for use as pesticide delivery agents were performed by many researchers [20-24]. Petroleum derived polymers with

Table 1: Evaluation of LC_{50} and LC_{95} for Three Stored Product Pests of Pellet in 24 h

					LC_{50}		LC_{95}	
Insect	Intercept+5	Slob ± SE		Sig(df=2)	Upper	Lower	Upper	Lower
Challosobruchusmaculatus	11.18	3.48±0.46	10.64	0.005	0.017		0.050	
					0.022	0.013	0.065	0.042

Table 2: Evaluation of LC_{50} and LC_{95} for Three Stored Product Pests of Phostoxin in 24 h

					LC_{50}		LC_{95}	
Insect	Intercept+5	Slob ± SE	Chi Square	Sig(df=3)	Upper	Lower	Upper	Lower
Challosobruchusmaculatus	6.27	1.40±0.32	0.67	0.879	0.124		1.852	
					0.094	0.221	0.653	28.573

Table 3: Analysis of variance the effect of Phostoxin at five concentrations on the *Challosobruchusmaculatus* by 24 h after treatment

Source	Sum of Squares	df	Mean Square	F	Observed Power
Intercept	19806.864	1	19806.864	7236.450**	0.0001
Concentrations	862.598	4	215.650	78.788**	
Error	27.371	10	2.737		
Total	20696.833	15			

Table 4: Significance of mean mortality percentages of *Challosobruchusmaculatus* due to Phostoxin using Tukey subset for alpha = 0.01 multiple range tests (One Way ANOVA)

	Subset		
Concentrations	1	2	3
1	25.3055		
2	31.0703	31.0703	
3		36.2712	
4			43.0869
5			45.9565
Sig.	0.011	0.021	0.282

Table 5: Analysis of variance the effect of Pellet at four concentrations on the *Challosobruchusmaculatus* by 24 h after treatment

Source	Sum of Squares	df	Mean Square	F	Observed Power
Intercept	17587.632	1	17587.632	3963.645**	0.0001
Concentrations	3575.920	3	1191.973	268.630**	
Error	35.498	8	4.437		
Total	21199.050	12			

Table 6: Significance of mean mortality percentages of *Challosobruchusmaculatus*due to Pellet using Tukey subset for alpha = 0.01 multiple range tests (One Way ANOVA)

	Subset		
Concentrations	1	2	3
1	19.8855		
2	24.0460		
3		46.9131	
4			62.2900
Sig.	0.150	1.000	1.000

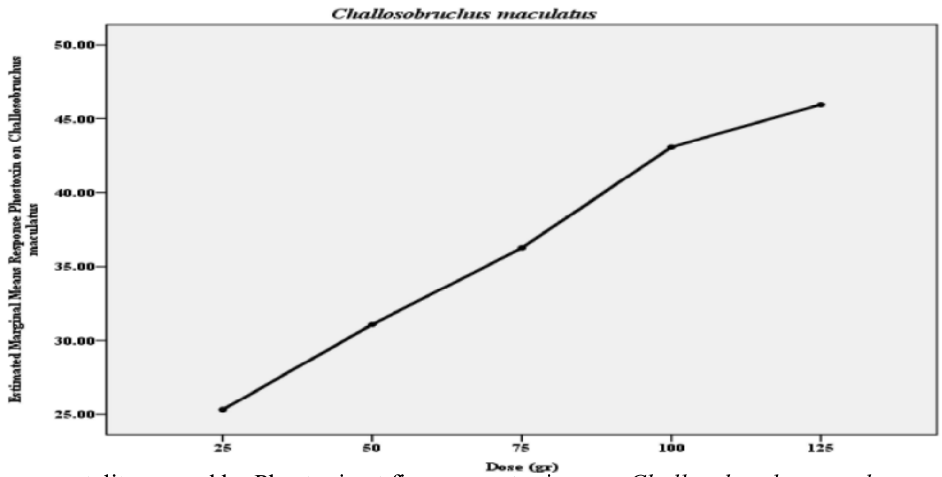

Fig. 1: Percentage mortality caused by Phostoxin at five concentrations on *Challosobruchusmaculatus*

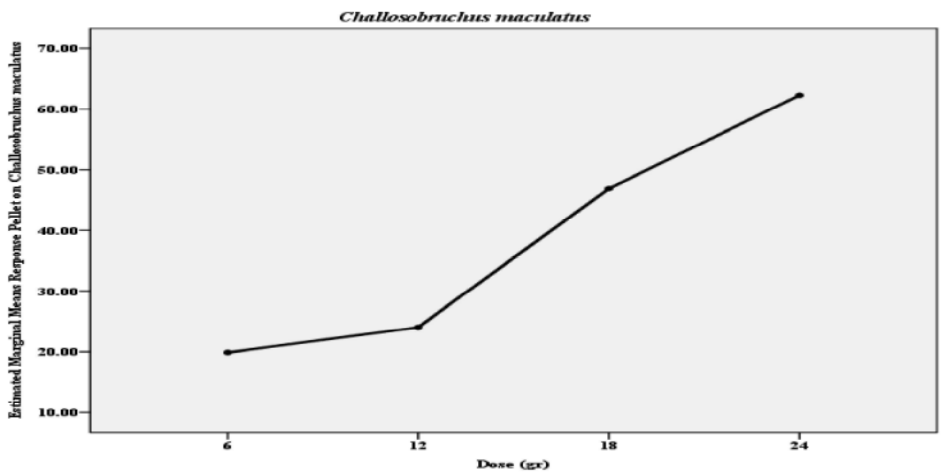

Fig. 2: Percentage mortality caused by Phostoxin at five concentrations on *Challosobruchusmaculatus*

biodegradable, non pollutant residues in environment and non toxic characteristics could be useful in pesticide delivery systems in agriculture [22]. However, application of natural polymers is more recommended [20, 21, 23, 24]. Produced formulations sizes were varied in different researches. Hu *et al*. [20] used 8 cm diameter spheres in their work. Singh *et al*. [24] wereproduced some beads (1.07-1.34 mm in size) and measured them by caliper. In present study 6 gram pellets thickness and diameter were measured by caliper and their average were 3±0.1 and 1±0.01 cmthat were recorded as pellets size. In this study, pellets wereproduced and expressed fumigant toxicity to *C. maculatus* to obtained results. we proposes that1, 8-Cineole and Acetone. Could be produced in pellet formulation and these pellets could have sufficient potency for insect pests control.Tablets taken as an adequate replacement forPhostoxintablets are introduced.The insecticidal efficacy varied with insect species,total of pellets. Physical loading of essential oils and their constituents on biodegradable polymers could be performed and their application in stores will be possible by this manner. Insecticidal efficacy of produced pellets should be evaluated against other stored products pests such as moths and mites in further studies. Also pellets could be produced by other biodegradable polymers such as poly(vinyl acetate) etc.

ACKNOWLEDGEMENTS

Authors are grateful to Urmia University for the grants that provided them to undertake this research.

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