# INVESTIGATION ON THE INSECTICIDAL EFFICACY OF NOVEL PELLET FORMULATION AGAINST STORED PRODUCTS BEETLES

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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 PVA, 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) of *Tribolium castaneum* (Herbst), *callosobruchus maculatus* (F.) and *Rhyzopertha dominica* (F.) under 28±2ºC and darkness in laboratory condition. $LC_{50}$ of 1 gram pellets against *T. castaneum* in 24 h and 48 h was 1.6 and 0.91 ml active ingredient per L air, respectively. $LC_{50}$ value for *R. dominica* and *C. maculatus* in 12 h was 0.7and 1.48 ml a.i./L air, respectively. Physical load of essential oils and their constituents could be performed on biodegradable polymers and their application in stores will be possible by this manner.

KEY WORDS: Controlled release, 1,8-Cineole, PVA, pellet formulation, fumigant toxicity.

Fumigation play the major role in insect pest elimination in stored products. 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 (Taylor, 1989; Bell & Wilson, 1995; Collins et al., 2002; Benhalima et al., 2004). In addition, there have been some arguments about the genotoxicity of phosphine (Garry et al., 1989).

Studies on fumigant toxicity of essential oils of plants and their constituents have been sharpened (Isman, 2006). Eucalyptus essential oil is one of the most important essential oils which has fumigant toxicity against insects. 1,8-Cineole (1,8-Cineole) is the most important compound of the genus Eucalyptus, and is largely responsible for its pesticidal properties (Batish et al., 2008).

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 (Han et al., 2009). 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 (Kenawy & Sherrington, 1992).

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 in second approach biologically active agent is heterogeneously dispersed or dissolved in a solid polymeric matrix which can be either biodegradable or non-biodegradable. Poly (vinyl alcohol) (PVA) is a water soluble polymer based on petroleum resources with unique properties such as good transparency, lustre, antielectrostatic properties, chemical resistance and toughness (Gohil et al., 2006). It has also good gas barrier properties and good printability. Its water solubility, reactivity, and biodegradability make it a potentially useful material in biomedical, agricultural, and water treatment areas (Chiellini et al., 2003). Because it is a biodegradable polymer and cheap to make Poly(vinyl alcohol) has been used as a polymer matrix for encapsulation of the pharmacological reactive agents (Fundueanu et al., 2007). Without a doubt, its use in crop mass does'nt has any health problem for human beings. In this research we loaded 1,8-Cineole, botanical constituent, on Poly(vinyl alcohol), biodegradable polymer, and evaluated insecticidal efficacy of produced pellet formulation, against three stored product pests.

## MATERIAL AND METHODS

1,8-Cineole with 154.25 g/mol molar mass and Assay (GC, area%) ≥ 98% was purchased from Merck (Darmstadt, Germany). Poly (vinyl alcohol) (Molar Weight: 72000 g/mol; hydrolysis mole: 98%) was purchased from Merck (Darmstadt, Germany).

All culturing insects were obtained from laboratory cultures from Urmia University, stored product pests incubation room. Rust red flour beetle (*Tribolium castaneum* (Herbst)), Cowpea beetle (*Callosobruchus maculatus* (F.)) and lesser grain borer (*Rhyzopertha dominica* (F.)), were reared on flour mixed with yeast (5% W/W), Cowpea and Wheat 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.

PVA and 1,8-Cineole mixture with various concentrations were prepared by Dry Mixing Method (DMM) in ice temperature on stirrer with 500 rpm for 4 h. Preparation of mixture was taken place in tight 100 ml balloons and mixing power provided with magnetic stirrer. Then prepared mixtures were transferred to FTIR pellet maker apparatus (Thermo Nicolet part No. 0016-035, USA) and pellets were produced by hydraulic pressure under 100 Kg (Fig. 1). Producing of a pellet from prepared mixture was taken about 1 min of manufacturing time. Average of ten 1 gr pellets diameter and thickness were measured by caliper that were 1.3±0.1 and 0.93±0.01 cm, respectively.

Assessment of fumigant toxicity of pellets without grain was carried out with 20 adults (1-3 days old) exposed to 250 ml glass jars sealed with screwed metal caps fitted with nylon and adhesive tape. Volume of each glass jar was measured by the amount of water it could contain. Preliminary trials in 24 h were undertaken on *T. castaneum* adults (1-3 days old), to determine optimum weight of pellets. Pellets were placed in containers (5 cm in length and 3 cm in diameter) in each glass jar; hence, controls had no pellet in their containers. 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. Four weights (0.25, 0.5, 0.75 and 1 gram pellets) with 5 concentrations (0.5, 1, 1.5, 2 and 2.5 ml active ingredient in 5 gr PVA) were used as factors.

According to preliminary trial results, one gram pellets were used as Experimental weight in original fumigant toxicity tests. Exposure time was 24 and 48 h for *T. castaneum* (because of its resistance against released 1,8-Cineole, and pellets limited capacity for attraction of active ingredient) and 12 h for *R. dominica* and *C. maculatus*. Insects 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 weight caused mortality data, were altered in $\arcsin\sqrt{x}$ then were analysed (FACTOR) by using the MSTAT C software. The dose-effect experiments were arranged by randomized complete design and the data were analysed with analysis of variance (ANOVA) by using the MSTAT C software. The $LC_{50}$ and $LC_{95}$ values were calculated by probit analysis (SPSS, 16). At the end we added 5 to all SPSS obtained intercepts.

## RESULTS AND DISCUSION

The fumigant activity of 1,8-Cineole has been evaluated against similar insects (Prates et al., 1998; Lee et al., 2004; Stamopoulos et al., 2007; Abdolmaleki et al., 2010). Obtained results about susceptibility of experimental insects to 1,8-Cineole, confirm prior researchers findings (Lee et al., 2002; Lee et al., 2004). In present study higher values of $LC_{50}$ and $LC_{95}$ were estimated for 1,8-Cineole, because a trace of 1,8-Cineole remained in polymer matrix in 24 h exposure time. We have obtained significant reduction ($P<0.01$) for $LC_{50}$ and $LC_{95}$ values on *T. castaneum* adults in 48 h in comparison with 24h to confirm this claim.

Preliminary test for determination of optimum pellets weight showed that insecticidal activity varied in all cases, with different pellet doses ($P<0.01$) (Table 1). Significant increase of mortality was observed in adults with increasing pellet weights ($P<0.01$). According to preliminary trials, 1 gram pellets had significantly higher effect than other weights thus we used these pellets in our main experiments (Fig. 2).

Fumigant toxicity of 1 gram pellets without grains against each of experimental species in 12 h (against *T. castaneum* in 24 and 48 h), is shown in table 2. Results indicates that *R. dominica* and *T. castaneum* had lowest and highest resistance against 1,8-Cineole and PVA based pellets, respectively. A significant increase of adults mortality was observed in all cases with increasing doses ($P<0.01$).

Fumigation containers volume were varied (e.g. 250, 500, 710 and 9000 ml) between different works (Keita et al., 2000; Lee et al., 2002; Lee et al., 2004; Stamopoulos et al., 2007; abdolmaleki et al., 2010). In this research we used 250 ml glass vials that have similarities to other fumigation vials in prior works (Lee et al., 2002; Lee et al., 2004).

Application of controlled release formulations in agriculture and study on different polymers for use as pesticide delivery agents were performed by many researchers (Hu et al., 1998; Jana et al., 2001; Riyajan & Sakdapipanich, 2009; Singh et al., 2009 a,b). Petroleum derived polymers with biodegradable, non pollutant residues in environment and non toxic characteristics, could be useful in pesticide delivery systems in agriculture (Riyajan & Sakdapipanich, 2009). However, application of natural polymers is more recommended (Hu et al., 1998; Jana et al., 2001; Singh et al., 2009a,b).

Produced formulations size were varied in different researches. Hu et al. (1998) used 8 cm diameter spheres in their work. Singh et al. (2009b) were produced some beads (1.07-1.34 mm in size) and measured them by caliper. In present study 1 gram pellets thickness and diameter were measured by caliper and their average were 0.93±0.01 and 1.3 mm that were recorded as pellets size.

In this study, pellets were produced and expressed fumigant toxicity to *T. castaneum, C. maculatus* and *R. dominica*. According to obtained results we propose that 1,8-Cineole could be produced in pellet formulation and these pellets could have sufficient potency for insect pests control. The insecticidal efficacy varied with insect species, dose of pellets, weight of pellets and exposure time.

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 starch and poly(vinyl acetate) etc for reach to cheaper to make pellets.

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Table 1. The results of ANOVA belonging to dose and weight of pellets.

| Source of variations | df | Mean square | F | P |
|---|---|---|---|---|
| Pellets dose | 4 | 11527.55 | 460.3 | 0.00 |
| Pellets weight | 3 | 7609.31 | 405.12 | 0.00 |
| Dose× weight | 12 | 306.48 | 4.08 | 0.00 |
| Error | 40 | 250.43 | | |
| Total | 59 | | | |

**: Indicate significant difference at $P< 0.01$, CV= 7.07 %.

Table 2. $LC_{50}$ and $LC_{95}$ values of 1 gram pellets fumigant toxicity.

| species | $\chi^2$ | slope | *P*(sig.) | $R^2$ | *$LD_{50}$ (lower bound –Upper bound) | *$LD_{95}$ (lower bound- Upper bound) |
|---|---|---|---|---|---|---|
| *T. castaneum* | 0.664 | 3.225 | 0.00 | 0.99 | 1.6 | 5.2 |
| (24 h) | | | | | (1.43-1.79) | (3.95-8.30) |
| *T. castaneum* | 0.481 | 5.489 | 0.00 | 0.99 | 0.91 | 3.38 |
| (48 h) | | | | | (0.81-1.04) | (2.45-5.90) |
| *C. maculatus* | 0.640 | 2.937 | 0.00 | 0.98 | 1.48 | 5.38 |
| (12 h) | | | | | (1.32-1.68) | (3.76-11.27) |
| *R. dominica* | 0.552 | 3.276 | 0.00 | 0.99 | 0.70 | 2.22 |
| (12 h) | | | | | (0.62-0.78) | (1.71-3.58) |

* : Values are in ml a.i./L air

Fig. 1. produced pellets.

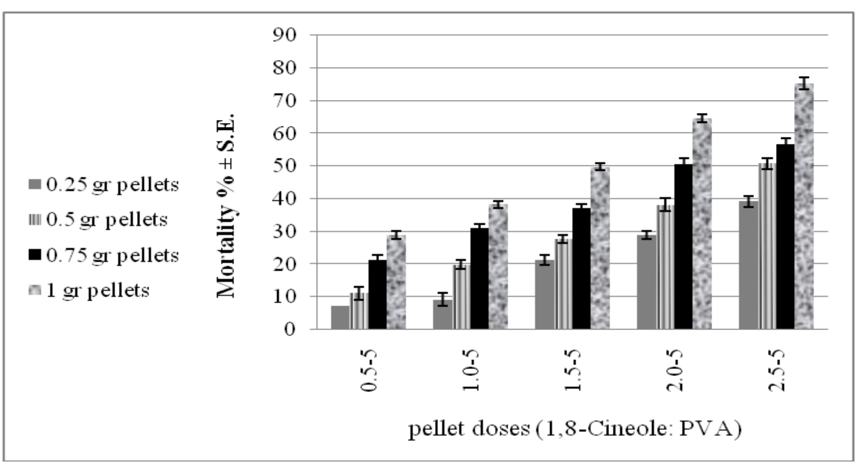


Figure 2. Mortality% ± S.E. of *T. castaneum* adults, exposed to different pellet weights in 24 h.