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Comparative toxicity of Malathion nano-emulsions prepared under different conditions against *Sitophilus. Oryzae& Tribolium castaneum*

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Abstract

The nano-emulsion (NEs) is heterogeneous and self-emulsifying colloidal system dispersed in a liquid medium, the small size of the internal phase allows the system to overcome the problems related to the gravity force, that lead to avoiding creaming or sedimentation phenomena. Also, the low surface and interfacial tensions provide pest spreading and penetration of the active compounds thus may lead to increase the efficacy of the active ingredient compared to the micro-emulsion. Both the stored products insects, the rice weevil *Sitophilus. Oryzae* L. and the red flour beetle, *Tribolium castaneum* are very important as causing a lot of damage to many crops and other products. We aimed in this study to evaluate the toxicity of two nano-emulsions of Malathion against *Sitophilus. Oryzae* L. and *Tribolium castaneum*, which still used till now in Egypt for the control of stored products insects, these nano-emulsions are prepared under different conditions as subjected to different sonication power,50 and 75 KHz then named M50 and M75 respectively, M50 droplet size varied from 206.2 to 290 nm. While, M75 droplet size varied from 93.60 to 94.30 nm. There is no degradation on Malathion molecule was observed after either sonication powers 50 or 75 kHz as no observed changes in the retention time. Then examined against the both insect using film technique compared to commercial Malathion (EC57%). Our results indicated that M75 was more toxic than M50 and both were more toxic than the commercial Malathion. Potentiation factor of M50 and M75 were 2.11 and 3.75 fold against *T. castaneum* and 1.54 and 2.47 fold against *S. Oryzae.* And this may be results from the difference in particle size in the prepared nano-emulsions.

Keywords: stored products, Malathion, nano-emulsions, *Sitophilus. Oryzae Tribolium castaneum*

Introduction

There are large losses in the production of stored grain due to their host insects in developing countries. This loss reaches 5-10% in temperate zones and 20-30% in the tropical zones (Rajendran. S and V. Sriranjini, 2008) [25]. The damage that result in the infestation with the Stored product pests not only limited to the quantitative loss, but also includes a change in product quality due to the contamination with insect feces, fragments, webbing and also its metabolites (Nasab, 2009) [22].

One of the most insects of stored grain is the rice weevil *Sitophilusoryzae (*L*).* (Coleoptera: Curculionidae). As both the adult and larva stages are feeding on whole grains. It damage whole wheat and many other grains, causing reduce both quality and quantity of stored products worldwide (Madrid *et al*, 1990) [18]. The second stored products pest is The red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae), (Robinson and W. Coleoptera, 2005) [27] which cause serious damage to many products as broken grain, cereals, milled grain products, dried pet food, pasta, chocolate, nuts, and cereals previously infected with insects (Via, 1999) [35].

The most control method that used for stored product pests control is Synthetic pesticides which are characterized by their rapid effect, ease of handling, application and storage.

The continuous application of these insecticides may cause many problems include the hazardous residues in the products, insect resistance and handling risk (White NDG, and Leesch JG. 1995 [36]; Auamcharoen *et al*, 2012) [2]

The development of resistance to insecticides in many stored product has been reported in many countries like Egypt and other countries (Dyte, C.E. and D. Halliday, 1985 [10]; Prickett, 1987 [24]; Rassman, 1988 [26]; Irshad, and Jilani, 1990 [15]; Zettler, and Cuperus, 1990 [39]; Sayaboc, *et al*, 1992 [28]; Yao, and Lo, 1995 [37]; DARP, 2003 [9]; Benhalima, *et al*, 2004) [7] this resistance phenomena has been reported against many insecticides that belongs to many types of pesticide groups especially malathion which recorded 199.6 fold with *Sitophilus oryzae* (DARP, 2003 [9]; Attia *et al* 2020) [1] Most insecticides are poorly soluble organic compounds in water, like many pyrethroids, organophosphates. Significant amounts of organic Solvents. Such insecticides are typically used to dissolve in order to achieve efficient and consistent storage or mill application however, results in environmental emissions from toxic solvents, and raises farm workers' personal exposure to different organic compounds. Organic diluents are often much more costly than water (Margulis-Goshen *et al*., 2013) [19] Nanotechnology provides an enticing way of solving these problems. Accordingly, nanopesticides, which is refer to the use of nanotechnology in pesticides has attracted the interest of most researchers who work in the development of plant protection products to achieve their goal by developing new active ingredients with nanoscale dimensions, as well as the development of their formulation and delivery (Smith, *et al*, 2008 [30]; Yasur and Usha Rani, 2013 [38]; Benelli and Lukehart, 2017) [6]

Nanoemulsions consist of fine oil-in-water dispersions, having droplets covering the size range of 100–600 nm. Nanoemulsions, usually spherical, are a group of dispersed particles used for pharmaceuticals biomedical aids and vehicles that shows great promise for the future of cosmetics, diagnostics, drug therapies and biotechnologies.

The nano-emulsion (NEs) is heterogeneous and self-emulsifying colloidal system dispersed in a liquid medium (Kesrevani and Sharma, 2016) [16]. The characteristic which enhance some physicochemical properties, as the small size of the internal phase allows the system to overcome the problems related to the gravity force that lead to avoiding creaming or sedimentation phenomena. Also the low surface and interfacial tensions provide pest spreading and penetration of the active compounds (Gutiérrez, *et al*, 2008) [13]. The size of nano-emulsion drops is ranged between 20 to 200 nm. (Tadros, *et al*, 2004) [33]. This study aimed to study the toxicity of two nano-emulsions of malathion that prepared under different conditions compared to the conventional malathion EC against the rice weevil *Sitophilus oryzae* and The red flour beetle, *Tribolium castaneum*

Materials and Methods

Insect rearing

S. oryzae has been continuously reared in the laboratory for about ten years at Faculty of Agriculture, Alexandria University. Adults (2-3 weeks old) reared on whole wheat under conditions of 28 ° C ±1, RH of 70 ±5 and photoperiod L/D of 12:12hr (Strong, *et al*, 1967) [32]. *T. castaneum* was reared on wheat flour mixed with yeast (10:1, w/w), both in 1 L glass jars that were covered with a fine mesh cloth for ventilation. And all experimental procedures were carried out at 27 °C and 65.5% R.H. two weeks emerged adults were used in studies

Preparation of nano-emulsion

Preparation of nano-emulsion was conducted in The Environmental Studies And Pesticides Toxicology Lab, the oil-in-water nano-emulsion was formulated using malathion technical grade 95%, non-ionic surfactant (tween 80), organic solvent DMSO (Dimethyl sulfoxide) and distilled water. Initially, coarse emulsion was prepared by adding the organic phase containing pesticide, solvent and olive oil in ratios 1:3:1 (v/v/v) using a syringe to a conical flask that containing the aqueous phase which consist of the distilled water and the non-ionic surfactant, all this steps were carried out on the magnetic stirrer, then the prepared formulation was divided into two portions each one subjected to ultrasonic emulsification under different condition, the first portion subjected to a 50kHz Sonicator for 5 minutes (referred as M50), the second was subjected to a 75kHz Sonicator for 10minutes(referred as M75)

Characterization of the prepared nano-formulation.

The particle size and PDI of nanoemulsion formulation was determined using Zetasizer Nano ZS (Malvern Instruments, UK) at room temperature in the central lab at the faculty of pharmacology, Alex. University. All nanoemulsion samples were diluted to 10% before measurements with deionized water to reduce multiple scattering effects. The particle size was measured by the average of three measurements as diameter in nm

Studying the effect of the preparation process on Malathion decomposition

For this study both prepared nano-formulation were injected into HPLC and compared with the retention time of the standard Malathion which injected at the same time under the same conditions.

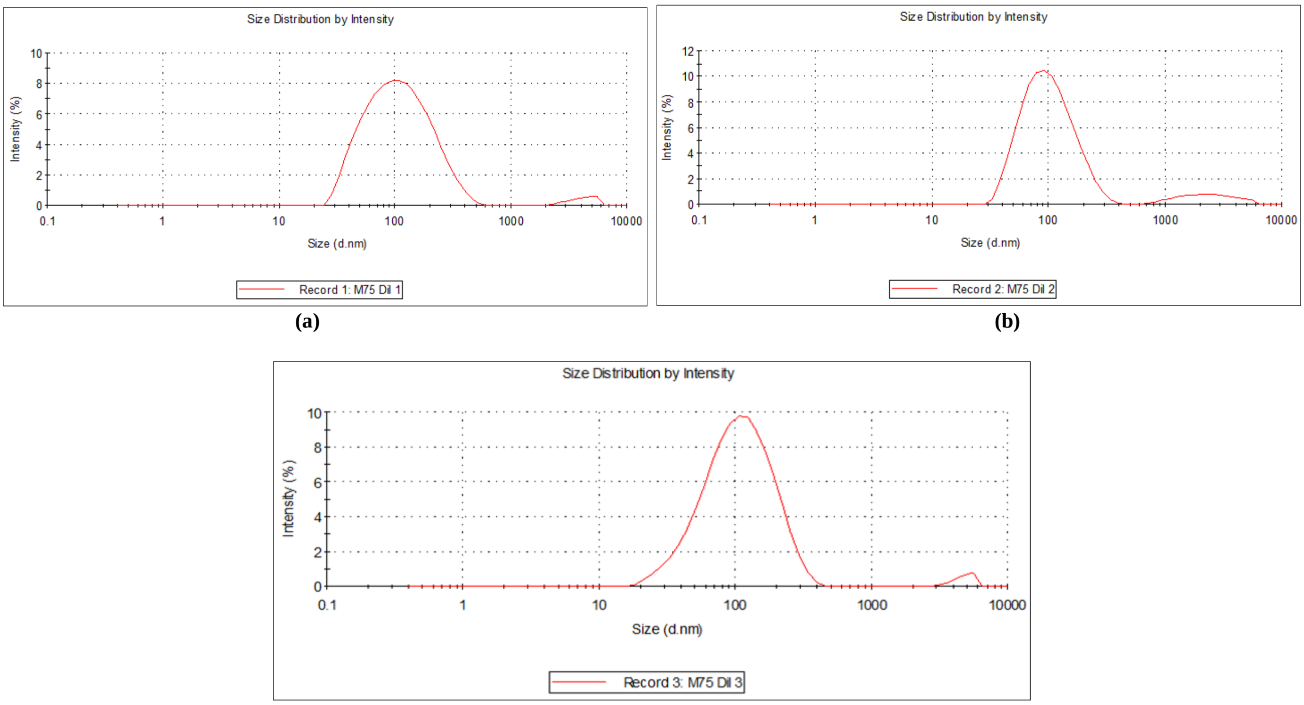
Contact toxicity bioassay using residual film technique:

The insecticidal activity of the tested formulations against the adults of S. *oryzae* and *Tribolium* was determined by direct contact application (Kim, *et al*, 2003 [17]; Usha Rani, and Rajasekharreddy, 2010) [34]. A series of dilutions of Malathion EC57%, M50 and M75 were prepared using acetone as a solvent. Aliquots of 1 ml of the dilutions were applied on the bottom of a glass Petri dish (9 cm diameter) to give different concentrations of each. After evaporation, 20 adults of tested insects were separately placed into each Petri dish. Control dishes with and without solvent were used. All treatments were replicated three times. Mortality percentages were recorded after 72 h of treatment and LC_{50} values were calculated according to Finney (Finney, 1971) [12]. Probit analysis was used to analyze the toxicity results to estimate the LC_{50} (Ldp line).

Result and Discussion

Characteristics of the nanoemulsion

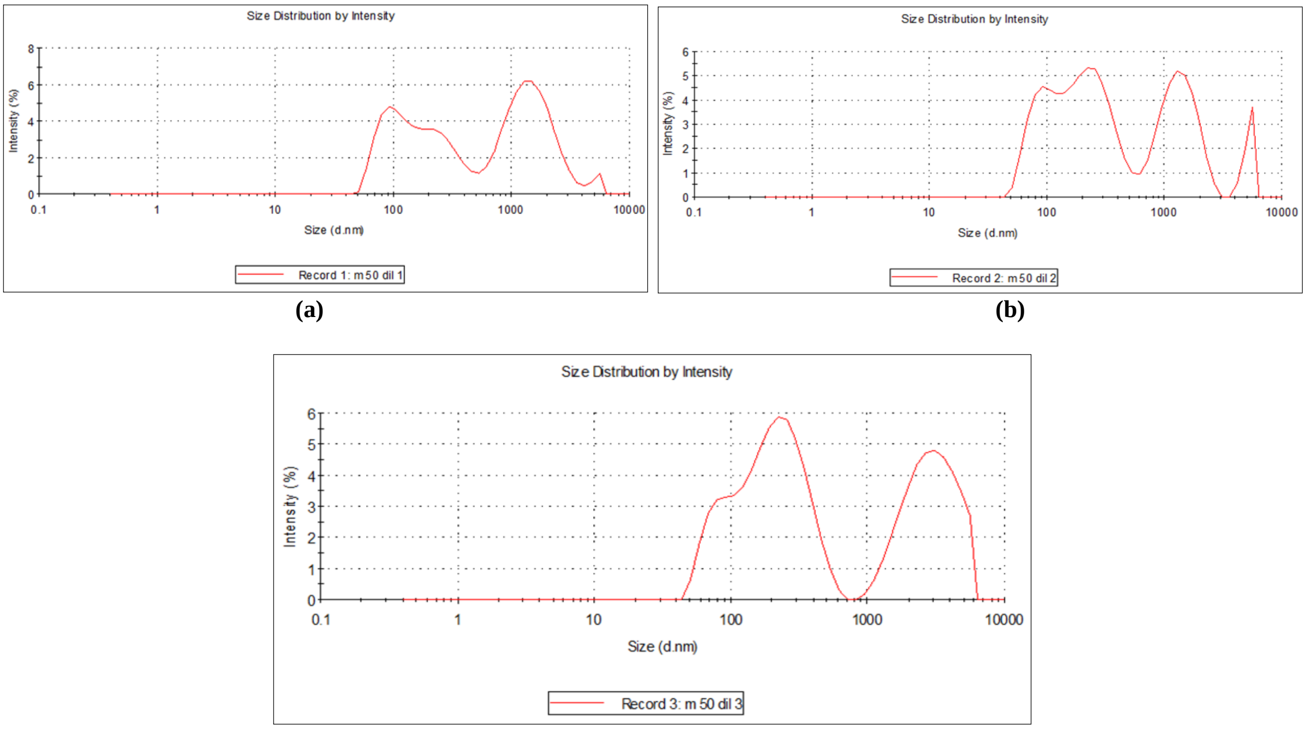
The data in figure (1, 2) indicate that, the decrease of particle sizes and polydispersity index (PDI) values by increasing sonication powers. M75 with droplet size varied from 93.60 to 94.30 nm with PDI values of 0.264–0.257. Compared to M50 the droplet size varied from 206.2 to 290 nm with PDI values of 0.774–0.879. Our results show that all emulsions were successful in their preparation in the nanometric size range. Our results are agreement with those who found that good nanoemulsion had droplets size between 20-200 nm (Ostertag, *et al*, 2012 [23]; Massoud, *et al*, 2018) [20]. Some insecticides known are organic compounds with poor water solubility. Development of nano-insecticides, including nanoemulsions solve this problem, increase water solubility of poorly water soluble substances and resulting in stable formulations without utilization of organic toxic solvents (Bordes, *et al*, 2009) [5]. They have two phases; the simplest is oil-in-water. These formulations show advantage properties such as varied viscosity, permeability, crystallinity, thermal stability, solubility and biodegradability. Nanoemulsions droplet containing diazinon as an insecticide had significantly affect with applied sonication power 75 kHz, ultrasonic time 20 min which, was the optimum conditions for provided more stable Nanoemulsions (Badawy, *et al*, 2017) [4]. Moreover, The emulsions subjected to ultrasonic emulsification for 15 min with sonication power of 10 kHz and pulses or cycles (9 cycle/sec) where, droplet size were in the range of 7.07–10.18 nm and a PDI of 0.249–0.620 (Badawy, *et al*, 2018) [3]. Furthermore The droplets size of nanoemulsion decreases with an increase in duty cycle; whereas pulsed ultrasound with proper intervals was more functional than continuous ultrasonication (Shahavi, *et al*, 2015) [29].



(a) (b)

(c)

Fig 1: Particle size distribution (nm) of Malathion nano-emusion (M75) as measured by three replicates (a, b, c)



(a) (b)

(c)

Fig 2: Particle size distribution (nm) of Malathion nano-emusion (M50) as measured by three replicates (a, b, c)

Degradation

Malathion degradation was estimated after sonication known it degraded by sonication or not. Results of HPLC revealed that, no degradation on Malathion molecule was observed after either sonication powers 50 or 75 kHz as no observed changes in the retention time was obtained figures (3, 4, 5).

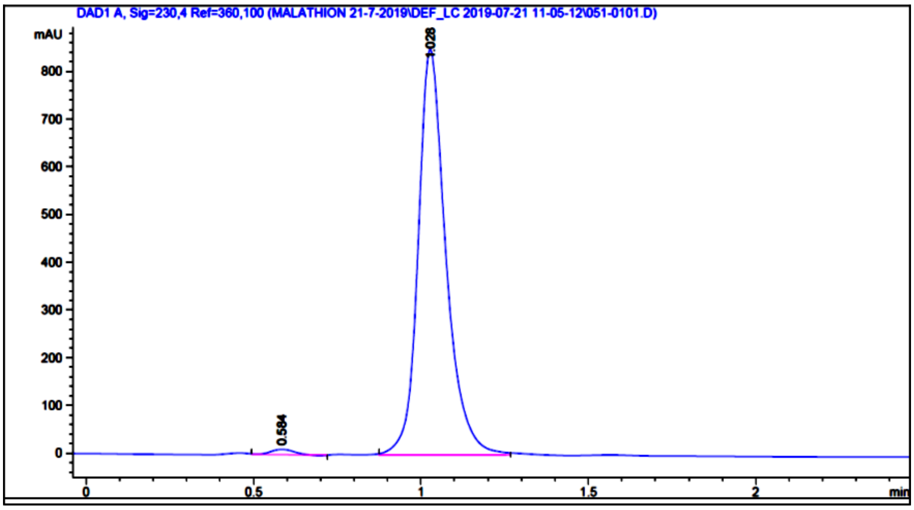


Fig 3: HPLC analysis for technical Malathion 95%, RT=1.028

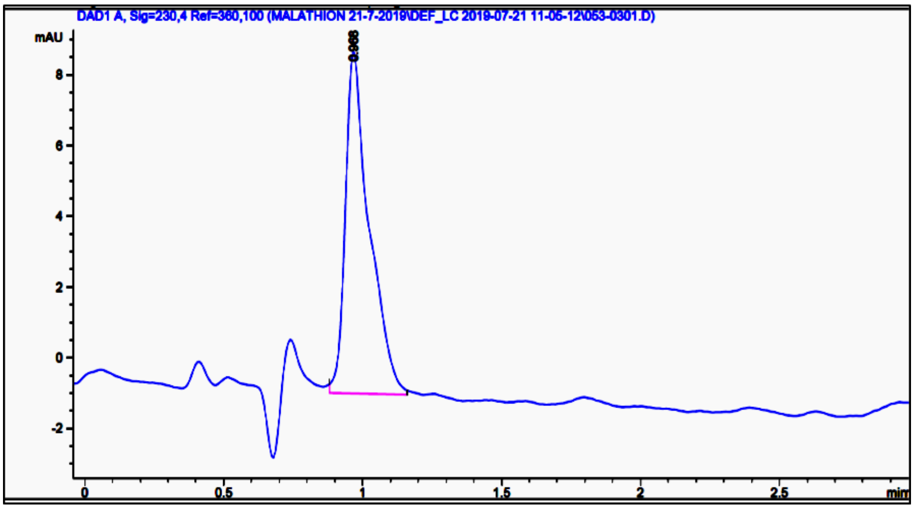


Fig 4: HPLC analysis for Malathion nano-emulsion (M75), RT=0.968

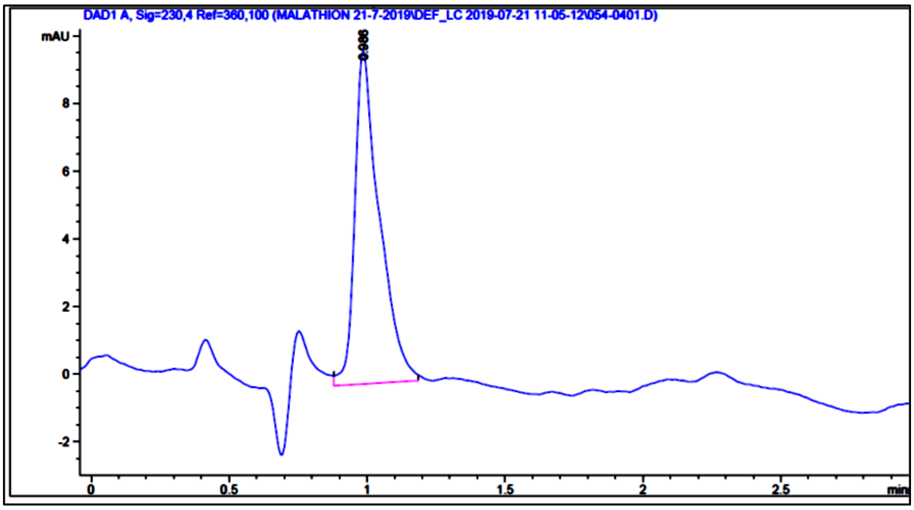


Fig 5: HPLC analysis for Malathion nano-emulsion (M75), RT=0.986

Contact toxicity bioassay using residual film technique

The results of insecticidal activity of Malathion formulations against adults *T. castaneu*m, and *S. oryzae* using contact assay after 72h of the treatment are shown in table (1). Malathion nanoformulation was more toxic than Malathion formulation. *S. oryzae* was highly susceptible than *T. castaneu*m, where LC_{50} of (nanoemalsetion 75 kHz) N.E2 (75) was 0.0023 mg/L followed by (nanoemalsetion 50 kHz) M50 0.0037 mg/L against *S. oryzae* but commercial formulation achieved lowest toxicity LC_{50} 0.0057 mg/L. Therefore, M75 and M50 were more effective 2.47 and 1.54 fold, respectively. *T. castaneu*m showed higher LC_{50} 0.026 and 0.044 mg/L for M75 and M50, respectively than Malathion where, LC_{50} was 0.093. Also, nano formulation increase Malathion toxicity about 3.57 and 2.11 fold to both M75 and M50 in comparison with Malathion formulation.

The biological activity of pesticides is an important criterion for the performance of pesticide nanoemulsions and has great significance for the control insects stored product Insect surfaces with waxy layers have poor hydrophilicity, thus, applied insecticides may easily fall off and have increased tendency to evaporate, seriously affecting their potency (Byun, *et al*, 2009) [8]. Other traditional pesticide formulations are relatively poor, which is not conducive to the absorption and penetration of the pesticides, seriously restricting their biological activity. The droplets size can significantly improve their adhesion and permeability on the target insects thereby increase the biological activity of pesticides and/or reducing their applying levels. A nanoemulsion with smaller droplets, excellent wetting performance, rapid diffusion, and penetration at the target surface is conducive to improve the biological activity of pesticides (Feng, *et al*, 2018) [11]. Moreover, our data are agreement with the whose obtained by many researchers which, mentioned that the high efficacy of nanoemulsion compared to the normal emulsion, the small droplet size of nanoemulsion that led to increased surface area and pesticidal activity compared to the normal emulsion (Sonneville-Aubrun, *et al*, 2004 [31]; Mossa, *et al*, 2019 [21]; Heydari *et al*, 2019 [14].

Table 1: Insecticidal activity of Malathion and its nano-formulations against adults *T. castaneum*, and *S. oryzae*.

T. castaneum					
Treatment (Formulation type)	**LC_{50}**	**Conf. limits**	**Slope ±SE**	**Q^2**	**P.f***
EC 57%	0.093	0.0322-0.2517	0.9383±0.0734	9.4482*(tab,7.8)	-
M(50)	0.044	0.0312-0.06131	1.1376±0.0907	2.4056 (tab,6)	2.11
M(75)	0.026	0.0187-0.0351	0.9879±0.0783	7.8535 (tab,6)	3.57
S. oryzae					
EC 57%	0.0057	(0.002-0.0124)	0.4280± 0.0919	0.0360(tab,3.8)	
M(50)	0.0037	(0.0021-0.006)	0.7578± 0.1008	0.3804(tab,3.8)	1.54
M(75)	0.0023	(0.0014-0.0036)	0.8901± 0.1101	2.7914(tab,3.8)	2.47

P. f*= potentiation factor

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