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Insecticidal activity of monocrotophos and pungam oil combinations for cotton pests management under laboratory condition

Actividad insecticida de las combinaciones de monocrotofos y pungam para el manejo de plagas de algodón en condiciones de laboratorio

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ABSTRACT

In this experiment, the joint action of crude pungam oil and a conventional insecticide monocrotophos was evaluated against *Pericallia recini* (Fab.) (Lepidoptera: Arctiidae) third stadium larvae and red cotton bug *Dydercus cingulatus* (Fab.) (Hemiptera: Pyrrhocoridae) adults under laboratory conditions. Monocrotophos and pungam oil were blended in 9:1, 8:2, 7:3, 6:4 and 5:5 proportions and used for the bioassay. Dose dependent mortality was not observed in the tested insects. *Dysdercus cingulatus* males showed higher resistance than females. Between two insect pests, lepidopteron larvae showed more resistance than hemipteran adults. Synergistic effects of monocrotophos with pungam oil were calculated by means of Sun's Co-toxicity Coefficients (CTC) and Finney's Synergistic Coefficient (SC), were recorded independent and antagonistic actions respectively. In conclusion, the pesticide was found to act additively with pungam oil under *in vitro* tested condition.

Key words*: Dysdercus cingulatus, Pericallia ricini,* conventional insecticide, essential oil, mortality.

RESUMEN

Se evaluó en condiciones de laboratorio los efectos conjuntos de aceite de pungam crudo y el insecticida convencional monocrotofós, sobre larvas de tercer estadio de *Pericallia recini* (Fab.) (Lepidoptera: Arctiidae) y adultos del chinche rojo del algodón *Dysdercus cingulatus* (Fab.) (Hemiptera: Pyrrhocoridae). Monocrotofos y aceite de pungam se mezclaron en las proporciones 9:1, 8:2, 7:3, 6:4 y 5:5 y se utilizaron para los bioensayos. La mortalidad dependiente de la dosis no se observó en los insectos ensayados. *Dysdercus cingulatus* machos mostraron mayor resistencia que las hembras. Entre las dos plagas evaluadas, las larvas del lepidóptero mostraron más resistencia que los adultos del hemíptero. Los efectos sinérgicos de monocrotofos con aceite de pungam se calcularon por medio de coeficientes de Sun Co-toxicidad (CTC) y Coeficiente sinérgica de Finney (SC), Se registraron acciones independientes y antagonistas, respectivamente. En conclusión, se encontró que el plaguicida actuaba aditivamente con aceite de pungam bajo condiciones ensayadas *in vitro*.

Palabras Clave*: Dysdercus cingulatus, Pericallia ricini, insecticida convencional, aceite esencial, mortalidad*.

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INTRODUCTION

Species of *Dysdercus* and *Pericallia* are important pests of cotton cultivars at various parts of the world. The red cotton bug *Dysdercus cingulatus* (Fab.) (Hemiptera: Pyrrhocoridae), both the adults and nymphs feed on the developing cotton boll and foliage seriously affects the crop yield and also the fruits quality thereby reducing its market value (Manzoor and Haseeb 2015). Monocrotophos was recommended for the management of *D. cingulatus* (Vennila *et al.* 2013). Another important pest of cotton is *Pericallia ricini* (Fab.) (Lepidoptera: Arctiidae) commonly known as castor hairy caterpillar, is a serious pest of cotton, castor, sunflower, gingelly, maize, ivy gourd, brinjal, sweetpotato, banana, Cucurbita, etc. (NBAIR 2018). Organophosphorus like monocrotophos is very useful to manage this pest since the 1980s (Pandey *et al.* 1980). Neem oil was also used for the *P. ricini* management (Chockalingam *et al.* 1987).

Monocrotophos a water-soluble organophosphate insecticide and acaricide used to control various pestiferous insects and mites on a variety of crops. However, monocrotophos is classified under highly hazardous pesticides by World Health Organization in 2004. Hence, monocrotophos is banned in United States of America, China, European Union, Indonesia, Argentina, Australia, South Africa and a few other nations. But in India, monocrotophos was banned for vegetables pest management (CIBRC, 2018). Available reports in India reveals that monocrotophos is toxic to human, birds, shrimps, crabs and moderately toxic for fishes (World Health Organization. 2009). Hence, it is essential to reduce the monocrotophos content in Indian pest management practice. As an initial step, in this research, we incorporate pungam oil, an important component of bio-intensive integrated pest management (BIPM) in order to reduce monocrotophos quantity.

Plant essential oils showed variety of bioactivity such as repellent, ovipositional, ovicidal and insecticidal activity against many insect pests (Srivastava *et al.* 2015) due to the presence of different bioactive principles. *Pongamia pinnata* L. [=*P. glabra* Vent. and *Derris indica* (Lamk) Bennet] has rich source of flavonoids (Pavela and Herda 2007) and alkaloids *viz.,* demethoxy-kanugin, gamatay, glabrin, glabrosaponin, kaempferol, kanjone, kanugin, karangin, neoglabrin, pinnatin, pongamol, pongapin, quercitin, saponin, b-sitosterol, and tannins (Kumar and Singh 2002)**.** Pungam oil showed insecticidal (Mariam and Chandramohan, 2000; Abo-El Seoud *et al.* 2005; Pavela and Herda, 2007; Adiroubane and Raghuraman, 2008; Mathur *et al.* 2012), acaricide (Ramaraju 2004) and anti-phytopathogenic (Narasimhan *et al.* 1998; Rajappan *et al.* 1999; Rajappan *et al.* 2001) activities. Further, pungam oil-based commercial insecticide PONNEEM also shows insecticidal activity (Packiam and Ignacimuthu 2012; Packiam *et al.* 2014).

Combined effects of oils and insecticides have been previously reported in the literature (Martín-López *et al.* 2006; War *et al.* 2011; Packiam *et al.* 2014). The hypothesis in this study was that the addition of pungam oil to the insecticide mixture will reduce the doses of monocrotophos, the record the best combinations, keeping mortality in defoliating and sucking insects. Moreover, so far there are no studies on the combined action of pungam oil and chemical insecticide monocrotophos. Considering the lacuna and also the importance of this eco-friendly approach, the present study was conducted in the laboratory to evaluate efficacy of pungam oil and monocrotophos mixture at various concentrations (0, 10, 20, 30, 40, 50 and 100%) against *D. cingulatus* adults and *P. ricini* third stadium larvae.

MATERIAL AND METHODS

Collection and maintenance of pests

Insects were collected at different stages of development of red cotton bug *D. cingulatus*, Tirunelveli and Thoothukudi districts, state Tamil Nadu, India. Insects were maintained on cotton seeds soaked in water in laboratory conditions at 29 ± 1.5 °C temperature, 75 ± 5% relative humidity and 11L:13D photoperiod. The eggs laid by the pests were maintained in a small plastic container (60 ml capacity). Once the red cotton bugs had reproduced for two generations, adults (289.3 and 532.2 mg for male and female, respectively) were selected for the bioassay. Sexed adult bugs used in all the experiments were about one day old.

Life stages of *P. ricini* were collected from castor and cotton agro-ecosystems of Tirunelveli Districts, Tamil Nadu and were maintained on castor leaves at room temperature (29±1.5 °C), relative humidity (70-80%) and photo period of 11L and 13D h in 1L capacity transparent plastic containers (7.0 x 15.0cm). Laboratory emerged adults of *P. ricini* (> 1 - day) (43.7 mg) were introduced separately into the chamber oviposition (43.7 cm x 35.0 cm) and fed with 10% solution sucrose, fortified with a few drops of mixture of vitamins (vitamin tablet Supradyn Multi) to improve the position. Batches of eggs were removed, maintained and incubated in petri dishes (1.5 cm x 9.5 cm). Larvae reared in the laboratory, to reach the third instar were inhibited food 6-12 hrs (452.7 mg) and then used for the experiment.

Pesticide preparations

Commercial formulations of monocrotophos (Jeyakrishna Pesticide Limited, Salem) and also pungam oil were purchased from the local market and utilized for the experiment. For monocrotophos and pungam oil, recommended field dose of 0.03% and 0.3% respectively was prepared using tap water. Monocrotophos was blended with pungam oil in five proportions: 1) 9:1 [10%-27µl monocrotophos + 3 µl oil + 5 µl teepol (0.01%) + 99.92 ml tap water]; 2) 8:2 (20%-24µl monocrotophos + 6 µl oil + 5 µl teepol + 99.92 ml tap water); 3) 7:3 (30%-21µl monocrotophos + 9 µl oil + 5 µl teepol + 99.92 ml tap water); 4) 6:4 (40%-18µl monocrotophos + 12 µl oil + 5 µl teepol + 99.92 ml tap water -); 5) 5:5 (50%-15µl monocrotophos + 15 µl oil + 5 µl teepol + 99.92 ml tap water) and used for the bioassay. For the preparation of mixture, in 1.5 ml effondoff tube, exact quantity of monocrotophos was taken using micro-pipette, and then desired quantity of crude pungam oil was added, gently and vigorously mixed thoroughly for 5 to 8 minutes. Add 5 µl of teepol, again mix gently against to get a clear solution. A 99.92 ml tap water was taken in a 100 ml capacity conical flask, pungam oil and monocrotophos mixture was added and used for the bioassay. UV-visible spectrum for pungam oil, monocrotophos and their mixture were recorded using UV-1800 (Shimadzu, Japan).

Toxicity bioassay

Two pieces of 2 x 2 cm size cotton leaves were dipped into the insecticide + pungam oil mixture solution for 10 minutes, air dried for 5-8 minutes and placed into the plastic vials (5.2 cm x 4.4 cm). Three *D. cingulatus* adult males were introduced. However, for *P. ricini* larvae, 1 g of castor leaves were dipped in insecticide + pungam oil mixture and the above procedure was followed, with each treatment represented by at least 6 replicates of 3 insects each. Control category was treated with tap water along with 5 µl teepol (0.01%). After 24 hours, unfed leaves, faecal pellets and dead animals were removed. Then the animals were maintained with fresh leaves up to their death. Dead animals were counted after 24, 48, 72 and 96 hours of exposure to the oil, monocrotophos, and their mixtures. Live insects were monitored for the survival and growth until reaching maturity. Leaf-dip method was followed for assaying toxicity according to standard protocol as previously described. Water alone was used as negative control and monocrotophos as positive control.

Joint action studies

The interaction between the various concentrations of pesticides were evaluated larvae, exposing toxic substances mixed together in their respective levels LC50, and was applied using the bioassay method of immersion of the leaf, recording the larval mortality. The joint action of monocrotophos + pungam oil mixtures at different concentrations was calculated in terms of Sun and Johnson co-toxicity factor (Sun and Johnson, 1960) to differentiate between potentiation, antagonism and additive effects using the following formula:

Co-toxicity factor = (O – E)/ E X 100

where, O is observed % mortality and E is expected mortality.

The co-toxicity factor differentiates the results into three categories. A positive factor of $\geq$ 20 indicates potentiation, a negative factor of $\leq$-20 indicates antagonism, and the intermediate values of >-20 to <20 indicate an additive effect. Since LC50 values obtained were estimated mathematically, they were tested again in the insect, in order to clarify the expected mortality.

Synergistic / antagonistic action

These tests were carried out to determine the synergistic / antagonistic action resulting from mixing of a definite quantity of insecticide at the concentration level causing no observed mortality (eg: LC0) with the plant oil essential at its LC50 value. By comparing the observed mortality with the expected mortality of the mixture (50%), the synergistic / antagonistic resulting factor (SF) could give an indication of the nature of the effect: SF>1 means synergism; SF<1 means antagonism; SF=1 means no obvious effect (Thangam and Kathiresan 1990). According to Mansour *et al.* (2010), a safety factor of ±0.05 was considered when ranking the synergism, SF>1.05 and antagonism S F<0.95.

Statistical Analysis

Corrected mortality was calculated as per Abbott correction formulae (Abbott, 1925) and then LC30, LC50 and LC90 values were derived using Probit analysis method (Finney 1971). Mean and standard error were calculated from the replication data. Mortality data were analyzed in a single factor analysis of variance (ANOVA) by SPSS version 20.0. Mortality data of monocrotophos and pungam oil treated animals were subjected to Students "t" test. All significance was expressed at 5% level.

RESULTS AND DISCUSSIONS

This was the first time that an essential oil of a plant was blended with monocrotophos and their toxicity was tested against cotton pests. Pungam oil consists of higher proportion of mono-unsaturated fatty acid (46%) and polyunsaturated fatty acid (33%) (Kumar and Kalidhar, 2003). When pungam oil was mixed with monocrotophos, a peptide linkage was formed and the following products were obtained (Figure 1). During our blending preparation, no precipitate was recorded for this combination and we recommend using this combination as pest management components of our farmers. Moreover, the mixture was more stable up to six months under the laboratory conditions.

UV-visible spectrophotometer analyses reveals λ max ranged 317 and 365 nm (365, 344 and 317 nm), 317 and 358 nm (358, 349, 333 and 317 nm) and 390 and 317 nm (390, 376, 356, 344, 337, 323, 317 nm) for crude pungam oil, monocrotophos and mixture respectively. In mixture the λmax common were 317, 344, 337 and 356 nm.

Monocrotophos at 0.03% caused 84.4% (t =1.740, P<0.05) and 80.0% (t =1.738, P<0.05) mortality for male and female, respectively within 96 h, whereas pungam oil (0.3%) caused less mortality [64.2% (t =1.741, P<0.05) and 70.7% (t =1.739, P<0.05) mortality for male and female, respectively]. Monocrotophos affects insect (Khalequzzaman and Nahar 2001) and mites (Kwon *et al.* 2010) nervous system which leads to their resistant against various insecticide. However, blending of pungam oil with MCP enhanced the mortality by 100.0% and 91.6% at 10 (df=10, 90; F= 0.750; p= 0.000520) and 20% (df=10,88; F= 3.000; P<0.00513) respectively in male *D. cingulatus* (Figure 2). It shows blending

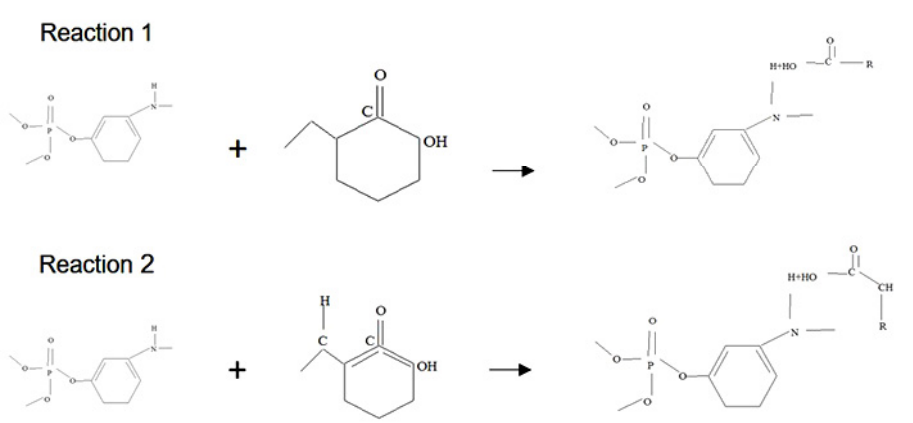

Figure 1. Reactions when pungam oil was mixed with monocrotophos.

of a small quantity of pungam oil increases the initial torpor, prolongs the duration of torpor, and results in a higher mortality of the insects. It was also reported that a combination of biological and chemical pesticide yields a promising alternative for insect pest management (Koppenhofer and Fuzy, 2003; Morales-Rodriguez and Peck, 2009). When pungam oil, neem oil, vijayneem and bio-silver nano particles were mixed with monocrotophos caused more mortality and weight loss of *Spodoptera litura* (Anbu Radhika and Sahayaraj 2014).

The best synergistic effect was recorded at LC50 value of 15.506% ($P<0.05$) in female *D. cingulatus* than in male (LC50 =24.464%; $P<0.005$). Table 1 which indicates that females were more resistant against the mixtures than males. Since female has more fat body, the insect needs more test material (monocrotophos and crude pungam oil mixture) to oxidise and died very quickly (12.4, 17.1 and 22.1% for 30, 40 and 50% mixtures) than males (33.712, 46.615 and 54.143% for 30, 40 and 50% mixtures). In *P. ricini* larvae, significant of *P. ricini* the mortality was higher in 10% (df= 10, 80; F= 5.729; $P<0.005$) and 20% (df=10, 87; F= 96.000; p= 0.0005) and 30% (df=10, 90; F=5.729; p= 0.005) concentrations at 96 hrs of the exposure. Synergistic impact was very low in *P. ricini* larvae (LC50 =28.752%; $P<0.05$). The comparison between the *D. cingulatus* and *P. ricini* showed that *D. cingulatus* adults have higher mortality (Figure 2) than *P. ricini* (Figure 3).

Prospectively pungam oil's synergic effects may be useful if mixed with a botanical neem insecticide in the azadirachtin bases (Kumar and Singh 2002; Srinivasa Rao *et al.* 2003), or with lower dosage of other synthetic insecticides (Vastrad *et al.* 2002; Rao and Dhingra 1997). Mortality results showed that combinations of mixture resulted in maximum activity more then the individual response of the pesticides. The mode of action of the essential oils or their constituents, as insecticides, remains unclear (Franzios *et al.* 1997). Further, they reported that many of them deter insects from feeding, while other have been proved to be neurotoxicant in their action or insect growth regulators, including analogs and antagonists of endogenous hormones.

We observed both the insecticidal and antagonistic activity of pungam oil and monocrotophos mixture. Antagonism is a diminution in the biological activity of a mixture of components compared with the individual activity of each component alone. We recommend the 9:1 (10%) mixture for both defoliating and sucking pests management. Sun and Johnson

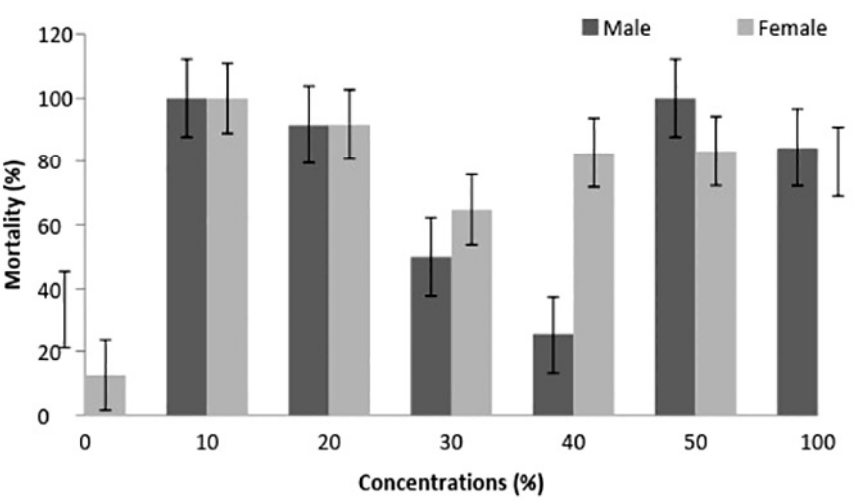

Figure 2. The mortality of *Dysdercus cingulatus* male and female exposed at 96 h to the mixture of monocrotophos and pungam oil at 90 + 10 (10%), 80 + 20 (20%), 70 + 30 (30%), 60 + 40 (40%) and 50 + 50 (50%) proportions.

(1960) and Mansour *et al.* (2010) synergistic factor analyses showed only antagonism both in *D. cingulatus* adults (male and female) and *P. ricini* larvae (Table 2) (Mansour *et al.* 2010). Results reveal that both pungam oil and monocrotophos either individually or combinedly interfered with the metabolic processes affecting the transformation of bioactives can be the mechanism of antagonism. Rao and Dhingra (2000) have reported that mixture of neem oil and cypermethrin or fenvalerate showed antagonistic impacts against *Spodoptera litura*

Table 1. The Probit analysis data of *Dysdercus cingulatus* adults and *Pericallia ricini* third stadium larvae treated with monocrotophos and pungam oil mixtures.

Pest(s)	Profit analysis data						
	LC_{30}	LC_{50}	LC_{90}	Slope	Chi-square	df	Significance (p)
D. cingulatus male	6.565	24.464	54.710	-1.686	216.824	5	0.005
D. cingulatus female	5.399	15.506	29.980	4.553	15.255	5	0.0005
P. ricini larvae	17.509	28.752	43.182	-6.945	5.726	2	0.05

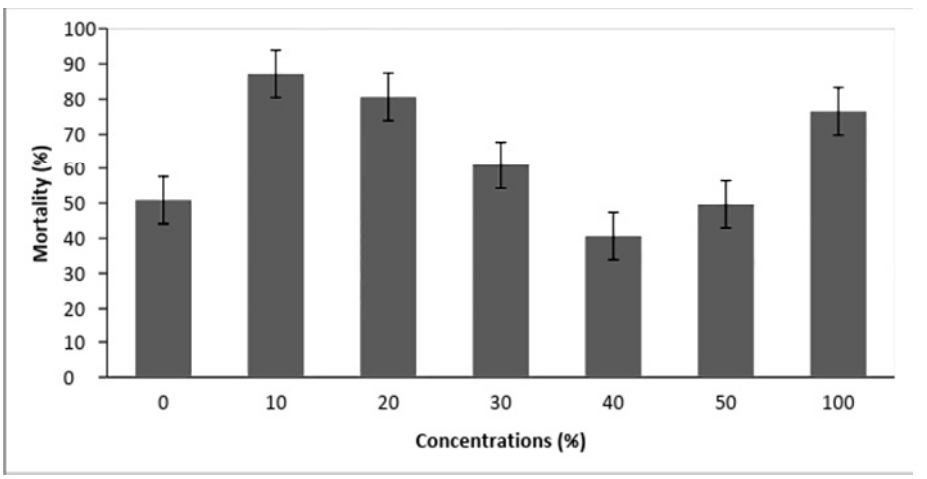

Figure 3. The mortality of *Pericallia ricini* third stadium larvae exposed at 96 h to the mixture of monocrotophos and pungam oil at 90 + 10 (10%), 80 + 20 (20%), 70 + 30 (30%), 60 + 40 (40%) and 50 + 50 (50%) proportions.

Table 2. Co-toxicity factor (CTF), Co toxicity coefficient (CTC) and Synergistic factor for *D. cingulatus* and third instar larvae of *P. ricini* treated with the mixture of monocrotophos and pungam oil at different ratios.

Pests	Co-toxicity factor (CTF)	Co-toxicity co-efficient (CTC)	Synergistic factor/ Antagonistic factor (SF/AF)
D. cingulatus male	-51.07 (AN)	0.824 (IA)	0.48 (AN)
D. cingulatus female	-68.98 (AN)	1.173 (IA)	0.31 (AN)
P. ricini larvae	-42.50 (AN)	2.839 (IA)	0.57 (AN)

AN=Antagonism; IA=Independent Action

as observed here. However, their coefficient analyses showed independent action need to have further research to confirm the findings.

CONCLUSIONS

We conclude that both compounds (oil pungam and monocrotophos) individually and in combination, led to the death in sucking insects (*D. cingulatus*) and also in defoliating (larvae, *P. ricini*), this was confirmed by co factors -toxicity and the co-toxicity coefficient. That means that action causes antagonism combined with pests, which is partly or totally opposed to the combination of the action of pests. The complete individual action on pests was confirmed by the co-toxicity coefficient. Furthermore, the content of monocrotophos can be reduced with the addition of essential oil. We can reduce the environmental impact through the use of biopesticides (oil pungam) combined with monocrotophos. However, the phytotoxicity must be studied before carrying out all tests under field conditions.

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