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Insecticidal effect of two novel pyrrole derivatives against two major stored product insect species

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ABSTRACT

Several pyrrole compounds exhibit insecticidal properties against a wide range of insect pest species. In the present work, the insecticidal effect of the two new pyrrole derivatives, ethyl 3-(benzylthio)-4,6-dioxo-5-phenyl-2,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrrole-carboxylate (3i) and isopropyl 3-(benzylthio)-4,6-dioxo-5-phenyl-2,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrrole-carboxylate (3k) were studied as stored-grain protectants (maize and barley) against two major stored-product insect species, the confused flour beetle, *Tribolium confusum* Jaquelin du Val (Coleoptera: Tenebrionidae) adults and larvae and the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) larvae at three doses (0.1, 1 and 10 ppm) and five exposure intervals (1, 2, 7, 14 and 21d). All *T. confusum* adults were dead at all doses on barley treated with 3i after 21d of exposure, while for 3k mortality was >92%. Progeny production was very low (≤1 individual per vial) at all doses for both pyrrole derivatives. Mortality of *T. confusum* larvae was not complete with any dose of both pyrrole derivatives but it exceeded 96% with 3k at 10 ppm after 21d of exposure on maize. For barley, all exposed larvae were found dead at all doses of both pyrrole derivates after 7d of exposure. Mortality of *E. kuehniella* larvae was complete at 1 and 10 ppm of 3i and all doses of 3k at 7d of exposure. After 14d of exposure, all *E. kuehniella* larvae were dead at 0.1 ppm of 3i. The results of the present study indicate that the tested compounds have elevated insecticidal effect against both species tested on certain combinations of dose, exposure interval and type of commodity.

1. Introduction

Stored product protection currently mainly relies on the use of convetional neurotoxic insecticides, i.e., organophosphates and pyrethroids (Arthur and Campbell, 2008; Daglish, 2008; Hertlein et al., 2011; Arthur, 2012; Kavallieratos et al., 2015). However, safer alternative compounds to the conventional ones have been evaluated in terms of efficacy, but also in terms of reduced risks for both human health and the environment. New compounds for this purpose need to fulfill two major requirements; First, any new compound that is intended to be applied on agricultural commodities should be evaluated as a plant protection product. Second, new compounds should combine high efficacy and low mammalian toxicity. In this context, newer compounds that have lesser mammalian toxicity and high insecticidal activity than insecticides of older chemistries have been registered for stored grain protection. For example, spinosad, which is derived from the metabolite of the actinomycete *Saccharopolyspora spinosa* Mertz and Yao (Actinobacteria: Actinomycetales) (Hertlein et al., 2011), has a 78 times higher LD_{50} than chlorpyriphos and 3.5 times higher LD_{50} than pirimiphos-methyl (USEPA, 1998; WHO, 2004; IUPAC, 2012). Another example is the insect growth regulator (IGR) methoprene, which is a juvenile hormone analog (JHA). This compound is

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considered safe and is used as aerosol, grain protectant or for surface treatments (Arthur, 2004; Daglish and Wallbank, 2005; Daglish, 2008; Arthur, 2010; Athanassiou et al., 2010a, b; Wijayaratne et al., 2012). Also, two more JHAs, hydroprene and pyriproxyfen, are registered for surface treatments against stored-product insect pest species in food facilities (Athanassiou et al., 2011; Arthur and Fontenot, 2012).

The pyrrole chlorfenapyr is non-neurotoxic and a recently developed compound with insecticidal properties. The compound has been registered for crack and crevice treatment against termites, cockroaches, and ants in the United States (Athanassiou et al., 2014). This compound causes oxidative phosphorylation in mitochondria, which disrupts the synthesis of ATP and has low mammalian toxicity (Hunt, 1996; Tomlin, 2000; McLeod et al., 2002). Chlorfenapyr has been evaluated with success for the control of several stored-product insect species in recent studies. For example, Guedes et al. (2008) and Athanassiou et al. (2014) found that chlorfenapyr was very effective against several psocid (Psocoptera: Liposcelididae) species when the compound was applied on concrete treated surfaces. Furthermore, Arthur (2013) showed that chlorfenapyr is effective against the red flour beetle, *Tribolium castaneum* (Hertz) and the confused flour beetle, *Tribolium confusum* Jaquelin du Val (Coleoptera: Tenebrionidae) when applied on concrete.

Pyrroles are five-member heterocyclic organic compounds characterized by a ring structure composed of four carbon atoms and one nitrogen atom. Other organic compounds linked with pyrrole derivatives are known to express biological activities, including insecticidal, antimicrobial and acaricidal activity. For example, Cantín et al. (1998) found that the 2-(3-Oxodecanoyl)-pyrrole and 2-(2-Methyl-3-oxopentanoyl)-pyrrole, which are analogues that are based on the pyrrolic metabolites of the fungus *Pennicillium brevicompactum* Dierckx (Eurotiales: Trichocomaceae), were highly effective against third instar nymphs of the milkweed bug, *Oncopeltus fasciatus* (Dallas) (Hemiptera: Lygaeidae) at 7.5 μg/cm^2 after 3d of exposure. Similarly, Cantín et al. (2000) found that the analogue N-Octanoyl-3-pyrroline, derived from the same fungus, caused complete mortality to third instar nymphs of *O. fasciatus* at 7.5 μg/cm^2 after 3d of exposure. The same compound showed 76.7% radial mycelial growth inhibition to the fungus *Colletotrichum coccodes* (Wallr.) S. Hughes (Glomerellales: Glomerellaceae). The pure compound, 5-(2, 4-dimethylbenzyl)-pyrrolidin-2-one, extracted from the marine actinobacteria *Streptomyces* VITSVK5 sp., caused complete mortality to larvae of the cattle tick, *Rhipicephalus microplus* (Canestrini) (Ixodida: Ixodidae), and to the mosquitoes *Anopheles stephensi* Liston (Diptera: Culicidae) and *Culex tritaeniorhynchus* Giles (Diptera: Culicidae) at 500 ppm after 24 h of exposure (Saurav et al., 2013). Recently, novel sulfanyl 5*H*-dihydro-pyrrole derivatives, with strong antioxidant activity (i.e., inhibition of lipid peroxidation and soybean lipoxygenase), have been synthesized (Georgiou et al., 2012; Oikonomou et al., 2015). However, there are no data available on the insecticidal properties of these substances. The objective of the present study was to investigate the insecticidal effect of two new pyrrole derivatives against two major stored product insect species, on two grain commodities.

2. Materials and methods

2.1. Insects

The insects used in the experiments were initially collected from Greek storage facilities and were reared at the Laboratory of Agricultural Zoology and Entomology, Agricultural University of Athens, at continuous darkness since 2014. *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) was reared on wheat flour at 26 °C and 60% relative humidity (RH). *T. confusum* was reared on wheat flour including 5% brewer's yeast (by weight) at 27 °C and 60% RH. Adults of *T. confusum* <2 weeks old and first instar-larvae of *T. confusum* or *E. kuehniella* were used in the tests. To obtain first instar larvae of *T. confusum* or *E. kuehmiella*, eggs of *T. confusum* or *E. kuehniella* were collected from flour by using a sieve of 60 mesh (250 micron, W.S. Tyler, Mentor, OH, USA), then placed in incubators at 26 °C and 60% RH, or 27 °C and 60% RH respectively, and larvae were collected, if hatched, after daily inspection.

2.2. Rearing media

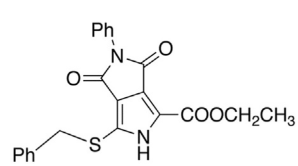
Clean and free of infestation and pesticides barley, *Hordeum vulgare* L. (var. Persephone) and maize, *Zea mays* L. (var. Dias), were used for the experimentation. The moisture content of the tested grain commodities was 11.1% and 10.8% for barley and maize respectively, as determined by a Dickey-John moisture meter (mini GAC plus, Dickey-John Europe S.A.S., Colombes, France) at the beginning of the tests.

2.3. Pyrrole derivatives

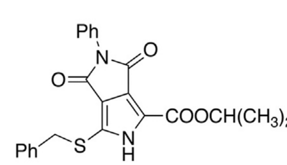
Two pyrrole derivatives, ethyl 3-(benzylthio)-4,6-dioxo-5-phenyl-2,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrrole-carboxylate and the isopropyl 3-(benzylthio)-4,6-dioxo-5-phenyl-2,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrrole-carboxylate, given the trivial name 3i and 3k, respectively (Fig. 1) (Oikonomou et al., 2015), were used in the study.

2.4. Bioassays

One-kg lots of barley or maize grain were placed in cylindrical glass jars and treated with the pyrrole derivatives at the following three doses: 0.1 ppm, 1 ppm and 10 ppm. The jars were shaken manually for 5 min to ensure equal distribution of the compounds on the entire grain. An additional 1 kg of untreated barley or maize was served as control. From each lot, three samples, of 10 g each, were taken and placed in small cylindrical glass vials (7 cm diameter, 12 cm height) with a different scoop that was inside each jar. The quantity of 10 g was weighed with a Precisa XB3200D compact balance (Alpha Analytical Instruments, Gerakas, Greece). The caps used on the vials had a 1.5 cm diameter hole in the middle, which were covered by muslin gauze, to allow sufficient aeration inside



Ethyl 3-(benzylthio)-4,6-dioxo-5-phenyl-2,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrrole-1-carboxylate



Isopropyl 3-(benzylthio)-4,6-dioxo-5-phenyl-2,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrrole-1-carboxylate

Fig. 1. Chemical structures of the pyrrole derivatives tested.