Agricultural Sci. J. 33 : 6 (Suppl.) : 318-324 (2002) **ว. วิทย. กษ. 33 : 6 (พิเศษ) : 318-324 (2545)**

กลไกการสร้างความต้านทานต่อสารกำจัดแมลงในมอดแป้งสายพันธุ์ต่างๆ

Insecticide Resistance Mechanisms in Various Strains of the Rust-red Flour Beetle (*Tribolium castaneum* Herbst)

สุรพล วิเศษสรรค์[1] วัชริยา ภูรีวิโรจน์กุล[1]
พิณทิพย์ กรรณสูตร และ Harley Rose[2]
Suraphon Visetson[1], Watchariya Purivirojkul[1],
Pintip Kannasutra and Harley Rose[2]

Abstracts

Insecticide-resistant mechanisms of 5 strains of the rust-red flour beetle (*Tribolium castaneum* Herbst), QTC4 (susceptible reference), WTC3 (fairy susceptible), CTC12, QTC285, และ QTC279 using *in vivo* impregnated filter papers at 25± 2 °C, 55 – 60%RH were trailed against cyfluthrin, malathion and fenitrothion. Synergists, PB, TPP, DEF and DEM, were added to the insecticides to reveal detoxification mechanisms in the beetle strains by means of median knock down dose (KD_{50}), resistance factor (RF) และ synergist ratio (SR).

After 24 hour exposure, the strain QTC279 gave the highest KD_{50} against cyfluthrin, malathion and fenitrothion ca. 21.45, 114.45 และ 6.645 g ai./L, respectively. Furthermore, QTC285 showed KD_{50} ca 0.17, 73.63 and 2.82 g ai./L, respectively. Hence the highest RFs value for QTC279 against the three nominal insecticides were 206-, 50- and 14 -fold, respectively. The strain WTC 3, CTC12 and QTC285 showed RFs against malathion were 5-, 10- and 30- fold, respectively. In addition, RFs against fenitrothion of the three strains were 7-, 6- and 14- fold, respectively.

Synergist studies with PB in cyfluthrin showed SRs for QTC279, QTC285, QTC12 and WTC3 were 39, 5.8, 2.6 and 6.0, respectively, indicating of monooxygenase was mainly responsible for cyfluthrin detoxification. Furthermore, after addition of PB, TPP, DEM and DEF to malathion, QTC285 and WTC3 gave SRs ca.15.1 and 24.4, respectively. This phenomenon reveals that apart from monooxygenase, esterase and glutathione-S-transferase may play a role in malathion detoxification in the two strains. Moreover, CTC12, QTC285 and QTC279 showed SRs between 1.9 – 2.4 after addition of PB, TPP and DEF but gave low SR after addition of DEM to fenitrothion. These final results indicated that monooxygenase as well as esterase probably the key factors involving fenitrothion detoxification in the strains. In contrast, the glutathione-S-transferase may not involve in the detoxification against this insecticide especially in the last three strains mentioned. The detailed mechanisms were discussed in the text.

บทคัดย่อ

จากการใช้วิธีการทดสอบกลไกการสร้างความต้านทานสารกำจัดศัตรูพืชของมอดแป้ง 5 สายพันธุ์ คือ QTC4 (susceptible reference), WTC3 (fairy susceptible), CTC12, QTC285, และ QTC279 โดยวิธี *in vivo* impregnated filter papers ที่อุณหภูมิ 25±2 °ซ. และ 55–60%RH กับสารไซฟลูธริน มาลาไธออน และเฟนิโทรไธออน ซึ่งใช้สารเสริมฤทธิ์ PB, TPP, DEF และ DEM เป็นเครื่องบ่งชี้กลไกการสร้างความต้านทานในมอดแป้งแต่ละสายพันธุ์ โดยพิจารณาค่า median knock down dose (KD_{50}), resistance factor (RF) และ synergist ratio (SR) ในการประเมินกลไกการสร้างความต้านทานต่อสารกำจัดศัตรูพืชทั้งสามชนิดในมอดแป้งดังกล่าว

จากการทดสอบพบว่าค่า KD_{50} ของมอดแป้งสายพันธุ์ QTC279 ที่มีต่อสารไซฟลูธริน มาลาไธออน และเฟนิโทรไธออน สูงที่สุดคือ 21.45, 14.45 และ 6.645 g ai./L ตามลำดับ ในขณะที่สายพันธุ์ QTC285 ให้ค่ารองลงมาเป็น 0.17, 73.63 และ 2.82 g ai./L ตามลำดับ ซึ่งทำให้ค่า RF สูงที่สุดได้จากสายพันธุ์ QTC279 ที่มีต่อสาร ไซฟลูธริน มาลาไธออน และ เฟนิโทรไธออน เป็น 206, 50 และ 14 เท่าตามลำดับ ในขณะที่สายพันธุ์ WTC 3, CTC12 และ QTC285 แสดงค่า RF ต่อสารมาลาไธออนเป็นปริมาณ 5, 10 และ 30 เท่า ตามลำดับเช่นกัน ส่วนค่า RF จากสารเฟนิโทรไธออนเป็น 1, 7, 6 และ 14 ตามลำดับ

จากการใช้สารเสริมฤทธิ์ เพื่อศึกษากลไกการทำลายพิษ สารกำจัดศัตรูพืชทั้งสามชนิดในมอดแป้งสายพันธุ์ต่างๆ พบว่าสายพันธุ์ QTC279, QTC285, QTC12 และ WTC3 ให้ค่า SR เป็น 39, 5.8, 2.6 และ 6.0 เมื่อมีส่วนผสมของสาร PB ลงในสารไซ

[1] ภาควิชาสัตววิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ บางเขน กรุงเทพฯ 10900
Department of Zoology, Faculty of Science, Kasetsart University, Bangkhen, Bangkok, Thailand 10900
[2] ภาควิชากีฏและโรคพืช คณะพืชศาสตร์ มหาวิทยาลัยซิดนีย์ รัฐนิวเซ้าส์เวลส์ ออสเตรเลีย
Department of Agricultural Entomology and Pathology, Faculty of Crop Science, The University of Sydney, N.SW., Australia

ฟลูธริน ซึ่งแสดงว่าเอนไซม์โมโนออกซิเจเนส เป็นกลไกที่สำคัญในการทำลายความเป็นพิษของสารไซฟลูธริน ส่วนสายพันธุ์ QTC285 และ WTC3ให้ค่า SR เป็น 15.1 และ 24.4 เมื่อมีการผสมสาร PB, TPP, DEM และ DEF ลงในสารมาลาไธออน ซึ่งแสดงว่านอกจากเอนไซม์โมโนออกซิเจเนส แล้ว เอนไซม์อื่นเช่นเอสเทอเรส และ กลูตาไธโอน-เอส-ทรานสเฟอเรส น่าจะมีส่วนในการทำลายความเป็นพิษของสารมาลาไธออน ในสายพันธุ์ทั้งสอง ในขณะที่ สายพันธุ์ CTC12, QTC285 และ QTC279 ให้ค่า SR ระหว่าง 1.9 – 2.4 หลังจากการผสมสาร PB, TPP และ DEF แต่ให้ค่า SR ต่ำเมื่อมีการเติมสาร DEM ลงในสารเฟนิโทรไธออน แสดงว่าเอนไซม์โมโนออกซิเจนเนส และเอสเทอเรส น่าจะเป็นผลต่อการทำลายพิษของมอดทั้งสามสายพันธุ์ แต่เอนไซม์กลูตาไธโอน-เอส-ทรานสเฟอเรส ไม่น่าจะให้ผลในการทำลายความเป็นพิษของสารเฟนิโทรไธออนในแมลงทั้งสามสายพันธุ์นี้ รายละเอียดของกลไกการสร้างความต้านทานได้กล่าวไว้ในเรื่องเต็ม

Introduction

Insecticide resistance is defined as follows: “resistance to insecticides is the development of an ability in a strain of insects to tolerate doses of toxicants which would prove lethal to the majority of individuals in a normal population of the same species” (Anonymous, 1975). The detection of resistance is base on insecticide susceptibility tests which are dosage-mortality experiments usually performed under laboratory conditions. There are three popular methods of insecticide testing in stored product insects, grain treated, topical and impregnated filter paper methods. The last method mentioned is the promising method. The method is simple, efficient in time, economical and no mechanization is required (Champ and Campbell-Brown, 1970). Through the recommendation of FAO (Anonymous, 1974), the detection and measurement of resistance in stored grain insects is based on insecticide impregnated filter papers. In addition, insecticide synergists represent a unique class of compounds. They are inactive when used by themselves. They increase the toxicity of an insecticide with which they are combined (Wilkinson, 1976). Consequently, in contrast to the largely inadvertent and unpredictable nature of most interactions, synergism of insecticides to insects is intentional and synergists are designed specifically for this purpose. When a metabolic resistance mechanism develops, it is often possible to negate that mechanism by use of an appropriate synergist (Casida, 1970). Metabolic studies with synergized insecticides help in the interpretation of the effects of synergist on the toxicity of insecticides. In this research the insecticides used were malathion, fenitrothion and cyfluthrin and synergists used to clarify the mechanisms of resistance in *T. castaneum* were PB, DEF, TPP, phrone and DEM. PB is a popular synergist which inhibits monooxygenases (Scott and Georghiou, 1986). DEM has been used to inhibit GSH S-transferases (Lamoreux and Rusness, 1987). It also inhibits DDT-dehydrochlorinase (Prabhaker *et al*., 1988). DEF (TBPT) has been used to study inhibition of esterase (Collins, 1990). Furthermore it has been reported that DEF can inhibit GSH S-transferases and monooxygenases (Prabhaker *et al*., 1988) respectively. TPP inhibits carboxylesterases specifically. Finally, phorone is believed that this synergist could interfere with the function of glutathione. Suggested in Collins (1990), all synergists were applied to the concentration of insecticides at the rate of 5% - 10% in the non volatile solvent (oil).

Materials and Methods

Beetles were supplied by Dr. Patrick J. Collins, Entomology Branch, Agricultural Research Laboratories, Department of Primary Industry, Queensland, Australia. There were 5 strains of *T. castaneum*. QTC4 is an insecticide-susceptible strain that has been unexposed to insecticides since 1965. QTC 285 is a malathion-specific resistance. QTC279 is a cyfluthrin-resistant strain. WTC3 was thought to be a susceptible strain from Western Australia. CTC12 is a multi-resistant field strain originating in Queensland. An impregnated-paper assay using the FAO recommended method No. 15 (Anonymous, 1974) together with modifications of Collins (1990) was used. The method depends on exposure of adult insect to insecticide impregnated paper. The insecticides, cyfluthrin, malathion and fenitrothion were dissolved in the solution mixture [acetone:hexane:Ondina 17 oil (non volatile solvent), 3:1.5:1] at a concentration of 250g/L in non volatile solvent. Using a 1 ml pipette, 0.5 ml aliquoats of the solutions were transferred to the filter papers (70 mm diameter). Using a progressively decreasing spiral to ensure even distribution. The papers were allowed to stand until the volatile solvents escaped. In case of insecticide plus synergist(s), the synergist(s) was added to the solvent mixture (3:1.5:1) at 100 g/L for PB, TPP and Phorone, and 50g/L for DEF and DEM (relative to non volatile solvent) before making the insecticide concentrations. Preliminary studies were undertaken with strain QTC4 in the determination of optimal synergist concentrations. Test insects were confined for 24-48 hours under fluorescent lighting at 22$\pm$2 $^{\circ}$C, 55 $\pm$ 5%R.H. The criterion of response to insecticide was knockdown (KD is knockdown concentration) defined as the inability of the insects to stand and walk in a coordinated manner. The mortality of

>0% and <100% corresponding with concentration of insecticide was used for computerized probit analysis. The resistant ratios (RF) were calculated from simple division of the KD_{50} resistant strain by the KD_{50} of the susceptible strain. Synergism ratios (SR) were calculated from dividing the KD_{50} of insecticide by the KD_{50} of insecticide and synergist mixture. All KD_{50}s were reported in terms of g active ingredient in nonvolatile solvent per liter (g/L).

Results and Discussions

1. Response to insecticides

Strain QTC279 was highly resistant to cyfluthrin giving a RF of 200> fold (Table 1). Although the RF in this study shows 20% less than Collins (1990) the major difference is in response of QTC4 (Collins KD50 = 0.045, my KD50 = 0.104). The different results may be due to both the criteria of personal error in the identification of death and different temperature effects (Subramanyam and Cutkomp, 1987). The slight resistance of QTC285 to cyfluthrin showed that the multi-resistance mechanism provides a weak resistance to pyrethroids (Collins, 1990). The strain CTC12 which showed no resistance to cyfluthrin in this experiment exhibited 2-5 fold RF for bioresmethrin and cismethrin in the treated grain method (Carter *et al*., 1975) and also somewhat tolerant to resmethrin, permethrin and phenothrin in the microcapillary method (Lloyd and Ruczkowski, 1980). These results indicate that strain CTC12 possess substantial cross resistance to unsynergised natural pyrethroids along with OP insecticides. The evidence of high malathion resistance in QTC285 and QTC279 was consistent with the grain treated method (Collins, 1990). Although these results can not be compared directly, (as a higher RF usually occurs using the treated grain method) there are similar trends in the response of the multi-resistant strain QTC285 with that of QTC279 to malathion. This suggests that OP insecticide resistance in QTC279 is most probably provided by the presence of the multi-resistance factor. I can assume that there are at least two resistance factors present in QTC279. Three strains of beetles, QTC12, QTC285 and QTC279 show low fenitrothion resistance. The resistance to fenitrothion of CTC12 was consistent with Carter *et al*. (1975) who showed that this strain exhibited ca. 6-fold RF for fenitrothion with the treated grain method.

Table 1 KD50s and resistance factors in various strains of *T. castaneum*

	QTC4	WTC3		CTC12		QTC285		QTC279	
Insecticides[a]	KD50[b] (±SD)	KD50[b] (±SD)	RF[C]	KD50[b] (±SD)	RF[C]	KD50[b] (±SD)	RF[C]	KD50[b] (±SD)	RF[C]
Cyflyuthrin	0.104a (0.016)	0.082a (0.005)	1	0.12a (0.02)	1	0.17a (0.027)	1.6	21.45b (5.51)	206
Malathion	2.31a (0.74)	12.12b (5.01)	5	26.41b (3.34)	10	72.63c (19.01)	30	114.45c (39.22)	50
Fenitrothion	0.48a (0.016)	0.39a (0.005)	1	3.41b (0.02)	7	2.82b (0.027)	6	6.64c (5.51)	14

[a] Means from 5 experiments, each of 4 replicates

[b] Means followed by the same letter within the same row are not significantly different, P=0.05.

[c] Resistance factor

2. Response to insecticides with synergists

Strain QTC279 shows an SR of 39 with PB (Table 2). The almost complete suppression of resistance in QTC279 suggested that monooxygenase was the major resistance mechanism. The evidence of incomplete suppression of resistance in this strain by PB was also reported by Collins (1990). This evidence indicates that some minor resistance mechanisms such as reduced penetration, enhanced metabolism, target site insensitivity or increased hydrolytic enzymes may be present in strain QTC279. The high synergism by DEF in strain QTC279 may not reveal the effect of esterases since it is possible that DEF may not block detoxification of esterase. It may be inhibiting monooxygenases (Plapp, in Collins, 1985) and GSH S-transferases (Prabharker *et al*., 1988). Therefore, the inhibition of resistance with DEF is not conclusive evidence of the involvement of esterases in the resistance. DEM showed no synergistic effect in all strains indicating that GSH S-transferases may not play any role in the detoxification of this insecticide. Moreover, DEM showed an antagonistic effect with strains CTC12 and QTC279. It can also be said that cyfluthrin would not be a substrate for GSH S-transferases. TPP had only a minor effect on strains

WTC3 and CTC12 indicating that carboxylesterase does not play a role in cyfluthrin detoxification. Also with the combination of TPP and PB in all strains the data indicate that carboxylesterase was unimportant in cyfluthrin detoxification. We assume that both esterases and monooxygenases are responsible for cyfluthrin resistance in QTC279 and the multi-resistant mechanisms in QTC285 provide low cyfluthrin resistance in this strain.

Table 2 KD_{50}s and synergist ratiosof five strains of *T. castaneum* to cyfluthrin with synergists.

	QTC4		WTC3		CTC12		QTC285		QTC279	
Synergists[a]	KD50[b] (±SD)	SR[C]	KD50[b] (±SD)	SR[C]	KD50[b] (±SD)	SR[C]	KD50[b] (±SD)	SR[C]	KD50[b] (±SD)	SR[C]
PB	0.022a (0.0005)	4.7	0.05a (0.04)	1.3	0.046a (0.016)	2.6	0.03a (0.012)	5.8	0.55b (0.11)	39
TPP	0.13a (0.022)	0.8	0.05a (0.022)	1.6	0.099a (0.005)	1.2	0.033a (0.004)	0.5	24.81b (1.10)	0.8
DEF	0.19a (0.0025)	5.2	0.029a (0.0027)	3.0	0.05a (0.004)	2.0	0.071a (0.005)	2.4	2.03b (0.33)	10.5
DEM	0.15a (0.03)	0.7	0.071a (0.027)	1.1	0.27a (0.027)	0.4	0.19a (0.01)	0.9	28.61b (6.51)	0.8
TPP+PB	0.027a (0.004)	3.8	0.05a (0.004)	1.6	0.044a (0.005)	2.7	0.11a (0.005)	1.6	0.55b (0.04)	39
TPP+DEF	0.044a (0.005)	2.4	0.013a (0.005)	6.0	0.044a (0.0005)	2.7	0.093a (0.016)	1.8	3.51b (0.38)	6.1
TPP+DEM+ DEF	0.016a (0.005)	6.3	0.019a (0.004)	4.3	0.049a (0.001)	2.4	0.09a (0.01)	1.9	2.92b (0.8)	7.2
TPP+DEM+ DEF+PB	0.013a (0.0005)	7.6	0.013a (0.0005)	6.0	0.038a (0.0004)	3.1	0.09a (0.0016)	1.8	0.27b (0.06)	78

[a] Means from 3-6 experiments, each of 4 replicates

[b] Means followed by the same letter within the same row are not significantly different, P=0.05.

[C] Synergist ratio

In general, synergism with TPP is supposed to be diagnostic of malathion-specific resistance caused by carboxylesterase. TPP did not, however, completely suppress malathion resistance in QTC279, QTC285 and CTC12 (Table 3). Other resistance mechanisms may play a part in malathion resistance in these strains. In other words, strains, WTC3, CTC12, QTC285 and QTC279 showed some levels of TPP and DEF synsegism. This is consistent with Prabhaker et al. (1988) who showed that esterases were associated with OP resistance (SR of DEF to TPP ca. 10). The highest SR of malathion resistance in this beetle was recorded by White and Bell (1988) who showed that in strain 81B the addition of TPP to malathion resulted in a SR of 400. Their experiment showed TPP completely inhibited carboxylesterase in the strain. Their result was also consistent with that of Subramanyam et al. (1989). Thus carboxylesterase appears to be involved in the metabolism of malathion in resistant strains of *T. castaneum*. The very slight synergism by DEM in strain CTC12, QTC285 and QTC279 indicated of GSH S-transferases may play a role in malathion resistance in these strains. Additionally, we believe that involvement of carboxylesterases as well as monooxygenases and GSH S-transferase are responsible for malathion resistance in strains CTC12, QTC285 and QTC279 and carboxylesterases are predominant in the detoxification of malathion in WTC3. Synergists could not completely suppress resistance to malathion in strains QTC285 and QTC279 indicating that acetylcholinesterase insensitivity as well as penetration resistance may be an additional resistance mechanism in these strain.

Table 3 KD_{50}s and synergist ratios of five strains of *T. castaneum* to malathion with synsergists.

	QTC4		WTC3		CTC12		QTC285		QTC279	
Synergists[a]	KD50[b] (±SD)	SR[C]	KD50[b] (±SD)	SR[C]	KD50[b] (±SD)	SR[C]	KD50[b] (±SD)	SR[C]	KD50[b] (±SD)	SR[C]
PB	2.82a (0.33)	0.8	11.01b (2.55)	1.1	17.61b (4.55)	1.5	74.82c (28.53)	0.9	83.61c (14.8)	1.4
TPP	1.54a (0.60)	1.5	1.12a (0.44)	11.0	8.25b (2.03)	3.2	14.81b (3.85)	4.9	79.51c (17.01)	1.4
DEF	1.54a (0.22)	1.5	0.49a (0.055)	24.4	13.22b (0.88)	2.0	10.45b (1.65)	6.9	24.22c (2.22)	4.7
DEM	3.8a (0.13)	0.6	72.01c (38.0)	0.1	21.43b (6.52)	1.2	68.21c (10.17)	1.1	73.15c (6.63)	1.6
TPP+PB	2.58a (0.16)	0.9	1.92a (0.27)	6.2	8.25b (0.77)	3.2	70.41c (42.93)	1.1	43.41c (8.83)	2.6
TPP+DEF	1.15a (0.11)	2.0	1.21a (0.44)	10.0	1.01a (0.27)	2.6	6.62b (0.99)	11.0	25.32c (7.84)	4.5
TPP+DEM+ DEF	0.77a (0.05)	3.0	0.49a (0.049)	24.4	7.72b (0.22)	3.4	4.78b (0.22)	15.1	20.35c (1.65)	5.6
TPP+DEM+ DEF+PB	0.55a (0.11)	4.2	0.47a (0..38)	24.4	7.71b (0.11)	3.4	8.25b (0.38)	8.8	18.7c (4.4)	6.1
TPP+Phorone	2.36a (0.38)	0.9	2.58a (0.16)	4.7	33.52c (6.53)	0.8	17.0b (0.005)	4.8	61.0c (27.01)	1.8

[a] Means from 3-6 experiments, each of 4 replicates

[b] Means followed by the same letter within the same row are not significantly different, P=0.05.

[C] Synergist ratio

The problem with the use of PB is that both synergism and antagonism can occur with OP insecticides. Only QTC279 showed some synergism with fenitrothion (Table 4). Perhaps one form of cytochrome P450 is activating the parent compound and at the same time, deactivating it. Perhaps there are different cytochrome P450s which are responsible for activation and deactivation. The same comments apply with other strains and with malathion as well as fenitrothion. Purification studies need to be undertaken with cytochrome P450s of the various strains of *T. castaneum* and only then will the situation be clarified.

We assume that the major mechanisms involved in resistance to fenitrothion in CTC12 and QTC285 are esterases and monooxygenase. In QTC279 monooxygenases are mainly involved with minor involvement of GSH S-transferases. These mechanisms did not account for all the resistance present. Insensitive acetylcholinesterases might also be involved.

To clarify the type of monooxygenases involved in OP insecticide metabolism, a synergist such as MKG264 could be usefully employed along with PB. If the beetles exhibited synergism with PB but not with MGK264, type 1 monooxygenase may be involved. If the beetles showed no synergism with PB but with MGK264, type 2 monooxygenases may be involved (Casida, 1970). If both synergists have an effect, type 3 monooxygenases may be involved (Wilkinson, 1971).

To clarify whether or not the rate of penetration and insensitivity of acetylcholinesterase are involved in resistant to fenitrothion and malathion in QTC285 and QTC279 the response of synergist such as thanite and organotins should be assayed with these insecticides. If the beetles exhibited synergism with thanite and/or resistance to organotins, penetration may be involved (Hoyer and Plapp, 1968). If no synergism by thanite is observed, insensitivity of acetylcholinesterase may be involved (Ayad and Georghiou, 1975).

Table 4 KD_{50}s and synergist ratios of five strains of *T. castaneum* to fenitrothion with synsergists.

Synergists[a]	QTC4		WTC3		CTC12		QTC285		QTC279	
	KD50[b] (±SD)	SR[C]	KD50[b] (±SD)	SR[C]	KD50[b] (±SD)	SR[C]	KD50[b] (±SD)	SR[C]	KD50[b] (±SD)	SR[C]
PB	1.04a (0.98)	0.4	2.91a (2.47)	0.1	4.33a (1.33)	0.8	3.24a (0.16)	0.8	2.86a (0.46)	2.3
TPP	1.26a (0.49)	0.4	0.41a (0.16)	0.9	3.24a (0.33)	1.1	1.04a (0.05)	2.7	6.25b (0.15)	1.0
DEF	0.18a (0.03)	2.5	0.42a (0.11)	0.8	2.36b (0.11)	1.4	2.42b (0.11)	1.1	5.01b (1.43)	1.3
DEM	0.42a (0.038)	1.1	0.52a (0.02)	0.75	4.12b (0.71)	0.8	2.64b (0.33)	1.1	4.73b (0.55)	1.4
TPP+PB	1.37a (0.27)	0.4	0.82a (0.38)	0.4	4.67b (1.37)	0.7	2.23b (0.71)	1.2	2.75b (0.63)	2.4
TPP+DEF	0.44a (0.05)	1.1	0.49a (0.05)	0.8	1.76b (0.38)	1.9	4.56b (1.15)	0.6	3.46b (0.33)	1.6
TPP+DEM+ DEF	0.44a (0.04)	1.1	0.44a (0.01)	0.8	3.83b (1.14)	0.9	5.54b (1.11)	0.5	3.85b (1.12)	1.9
TPP+DEM+ DEF+PB	0.27a (0.11)	1.7	0.38a (0.12)	1.0	3.82a (1.63)	0.9	4.18a (1.82)	0.6	2.75a (0.33)	1.7
TPP+Phorone	0.88a (0.16)	0.5	0.66a (0.12)	0.6	-	-	1.71a (0.005)	1.6	4.18b (0.88)	2.4

[a] Means from 3-6 experiments, each of 4 replicates

[b] Means followed by the same letter within the same row are not significantly different, P=0.05.

[C] Synergist ratio

Acknowledgement

We especially thank Dr. Patrick Collins and Dr. Andrew Kotze for providing various strains o*f T. castaneum* and we also gratefully acknowledge their guidance of his vast knowledge of insect culturing and selecting methods.

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