Correct computation of resistance ratio of an arthropod pest population from bioassay reliable and useful in insecticide and acaricide resistance management

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ASTRACT

The resistance ratio (RR) or resistance factor (RF) measures the level of resistance of a test population or strain of arthropod pest and is useful for monitoring and detection of resistance to insecticides and acaricides for resistance management. Two research teams working on insecticide resistance in the diamondback moth (DBM), *Plutella xylostella* (L.) in Ghana calculated the RR of a DBM population from bioassay by dividing the susceptibility of the DBM population by the recommended field concentration, a method which is erroneous and undermines insecticide and acaricide resistance management. The correct computation of RR is by dividing the susceptibility of the test population or strain of arthropod pest by the susceptibility of a fully susceptible strain of same species. From the correct calculation of RR, it is shown that the RR of a test population is greatly underestimated when it is calculated with the recommended field concentration. Thus, calculating the RR with the recommended field concentration does not permit the detection of resistance in its early stage of development because even when the method detects an insignificant decrease in susceptibility, there is already a significant accumulation of resistance genes in the population which renders insecticides and acaricides ineffective. When the RR is calculated correctly with a fully susceptible strain, the true resistance level of the test population is known and resistance research results are reliable and useful in the management of insecticide resistance development in arthropod pests.

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Introduction

In insecticide and acaricide resistance studies in agriculture and public health, bioassay allows monitoring, detection and characterization of resistance in arthropod pests (Ninsin *et al*., 2000; Ninsin & Miyata, 2003; Ninsin, 2003). When biochemical and molecular techniques are employed in resistance studies, bioassay is still useful, as it complements the biochemical and molecular techniques (Rauch & Nauen, 2003; Nyoni *et al*., 2011).

To determine the resistance level of an arthropod pest population from bioassay, the resistance ratio (RR), which is also known as the resistance factor (RF) is calculated. In order for RR from a bioassay to be reliable in resistance research and useful for resistance management, it is important that the RR of the pest population is calculated correctly. Two research teams working on insecticide resistance in the diamondback moth (DBM), *Plutella xylostella* (L.) in Ghana both calculated the RR of DBM population from bioassay by dividing the susceptibility of DBM population by the field recommended concentration (dosage or rate). Odhiambo *et al.* (2010) computed the RR

(denoted as fold resistance) as LC_{95} of field population divided by recommended dosage, while Botwe *et al*. (2012) computed RR (RF) as LD_{50} of field population divided by field recommended rate. The method used by Odhiambo *et al.* (2010) and Botwe *et al*. (2012) to calculate RR from bioassay is erroneous, and negatively impacts resistance research results and arthropod pest resistance management.

This review shows the correct computation of RR of an arthropod pest population from a bioassay and how the method by Odhiambo *et al.* (2010) and Botwe *et al.* (2012) negatively impacts resistance research results and management of insecticide and acaricide resistance.

Correct computation of RR

In order to understand how to calculate the RR of an arthropod pest population correctly from bioassay, the definition of resistance needs to be considered. The World Health Organization (WHO) (1957) defined resistance as the development of an ability in a strain of an organism to tolerate doses of a toxicant that would prove lethal to the majority of individuals in a normal (susceptible) population of the same species. Scott (1995) clarified that resistance is a heritable trait that was already expressed in at least some of the individuals in the population prior to the exposure to the toxicant. The definition of resistance by WHO (1957) and Scott (1995) indicate that before the exposure of a field population of arthropod pest to insecticides or acaricides, the population is susceptible, with a very high frequency of susceptible genes and very low frequency of resistance genes undetected by bioassay.

After insecticide application and selection of the resistance genes in the parent population, the frequency of the resistance genes in the progeny population increases so that the concentration of insecticide exerting the selection pressure kills fewer individuals in the progeny population than in the parent population (van Emden, 1974). As insecticide use is continued, the resistant-allele becomes sufficiently common in the population and the effectiveness of the insecticide is significantly reduced (Metcalf, 1989) because of a shift in the susceptibility of the arthropod pest population towards resistance. Thus, to determine the level of resistance attained by the population after exposure to the insecticide or acaricide with bioassay, the influence of the accumulated resistance genes on the effectiveness of the insecticide or acaricide would have to be compared with the influence of susceptible genes on the effectiveness of the insecticide or acaricide, and not the field recommended concentration.

The RR of an arthropod pest population which indicates the level of resistance would, therefore, be calculated as susceptibility (lethal concentration [LC] or lethal dose [LD]) of the test population or selected strain divided by LC or LD of a fully susceptible strain of same species (Ninsin *et al*., 2000; Ninsin, 2004a; Ninsin, 2004b; Ninsin, 2011) and expressed as:

$$RR = \frac{\text{LC of field population or selected strain}}{\text{LC of susceptible strain}}$$

where the reference susceptible strain is laboratory developed with full susceptibility to the insecticide or acaricide. From the above calculation of RR, resistance can be defined as a heritable decrease in the susceptibility of an arthropod pest population to an insecticide compared with a strain of same species that is fully susceptible to the insecticide. If a reference laboratory susceptible strain is not available for the calculation of RR, a field population of same species with high susceptibility to the insecticide or acaricide, indicating the presence of a high frequency of susceptible genes compared to other field populations, should be identified and used in place of the laboratory susceptible strain, as demonstrated by Antwi-Agyakwa *et al*. (2014).

Consequences of computing RR with field recommended concentration

Odhiambo *et al.* (2010) and Botwe *et al.* (2012) calculated the RR from bioassay with the field recommended concentration as follows:

$$RR = \frac{\text{LC (LD) of field population}}{\text{Field recommended concentration (dose/rate)}}$$

This method indicates that the field recommended concentration of an insecticide or acaricide would have to fail to control a field population of arthropod pest before resistance is considered to have developed in the population. The calculation of RR according to Odhiambo *et al.* (2010) and Botwe *et al.* (2012) with the field recommended concentration might have been inferred from a resistance definition such as that of IRAC (2012). Resistance is defined by IRAC (2012) as a heritable change in the sensitivity of a pest population that is reflected in the repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that pest species. However, using the resistance definition by IRAC (2012) as basis for calculating the RR with the field recommended concentration is erroneous, as explained above, and underestimates the RR values as shown in Tables 1-4 with DBM strains selected for resistance with four insecticides of different chemistries.

TABLE 1

Resistance ratio computations using LC_{50} of diamondback moth (DBM) population selected with concentrations of phenthoate 50% EC (KOBII-phenthoate selected strain)[a], LC_{50} of DBM susceptible strain (KOBII-NS strain)[a] and upper limit of recommended field concentration

KOBII-phenthoate selected strain LC_{50} (mg/l) (95% CI)	*KOBII-NS strain LC_{50} (mg/l) (95% CI)*	*KOBII-phenthoate selected strain LC_{50} (mg/l) (95% CI)*	*Upper limit of recommended field concentration (mg/l)*	*Upper limit of recommended field concentration (mg/l)*	*KOBII-NS strain LC_{50} (mg/l) (95% CI)*	*Resistance ratio*
610 (388–834)[a]	4.36 (3.63–5.40)[a]	-	-	-	-	140[ab]
-	-	610 (388–834)[a]	500	-	-	1.22[c]
-	-	-	-	500	4.36 (3.63–5.40)[a]	115[d]

[a] Cited from Ninsin (2004a)

[b] Resistance ratio = LC_{50} KOBII-phenthoate selected strain ÷ LC_{50} KOBII-NS strain

[c] Resistance ratio = LC_{50} KOBII-phenthoate selected strain ÷ upper limit of recommended field concentration

[d] Resistance ratio = Upper limit of recommended field concentration ÷ LC_{50} KOBII-NS strain

TABLE 2

Resistance ratio computations using LC_{50} of diamondback moth (DBM) population selected with concentrations of cartap 50% WP (KOBII-cartap selected strain)[a], LC_{50} of DBM susceptible strain (KOBII-NS strain)[a] and upper limit of recommended field concentration

KOBII-cartap selected strain LC_{50} (mg/l) (95% CI)	*KOBII-NS strain LC_{50} (mg/l) (95% CI)*	*KOBII- cartap selected strain LC_{50} (mg/l) (95% CI)*	*Upper limit of recommended field concentration (mg/l)*	*Upper limit of recommended field concentration (mg/l)*	*KOBII-NS strain LC_{50} (mg/l) (95% CI)*	*Resistance ratio*
238 (190–293)[a]	15.5 (12.5–19.4)[a]	-	-	-	-	15.4[ab]
-	-	238 (190–293)[a]	500	-	-	0.476[c]
-	-	-	-	500	15.5 (12.5–19.4)[a]	32.3[d]

[a] Cited from Ninsin (2004a)

[b] Resistance ratio = LC_{50} KOBII-cartap selected strain ÷ LC_{50} KOBII-NS strain

[c] Resistance ratio = LC_{50} KOBII-cartap selected strain ÷ upper limit of recommended field concentration

[d] Resistance ratio = Upper limit of recommended field concentration ÷ LC_{50} KOBII-NS strain

TABLE 3

Resistance ratio computations using LC_{50} of diamondback moth (DBM) population selected with concentrations of acetamiprid 20% SP (KOBII-acetamiprid selected strain)[a], LC_{50} of DBM susceptible strain (KOBII-NS strain)[a] and upper limit of recommended field concentration

KOBII-acetamiprid selected strain LC_{50} (mg/l) (95% CI)	*KOBII-NS strain LC_{50} (mg/l) (95% CI)*	*KOBII-acetamiprid selected strain LC_{50} (mg/l) (95% CI)*	*Upper limit of recommended field concentration (mg/l)*	*Upper limit of recommended field concentration (mg/l)*	*KOBII-NS strain LC_{50} (mg/l) (95% CI)*	*Resistance ratio*
512 (414–639)[a]	54.3 (44.4–67.1)[a]	-	-	-	-	9.43[ab]
-	-	512 (414–639)[a]	200	-	-	2.56[c]
-	-	-	-	200	54.3 (44.4–67.1)[a]	3.68[d]

[a] Cited from Ninsin (2004a)

[b] Resistance ratio = LC_{50} KOBII-acetamiprid selected strain ÷ LC_{50} KOBII-NS strain

[c] Resistance ratio = LC_{50} KOBII-acetamiprid selected strain ÷ upper limit of recommended field concentration

[d] Resistance ratio = Upper limit of recommended field concentration ÷ LC_{50} KOBII-NS strain

TABLE 4

Resistance ratio computations using LC_{50} of diamondback moth (DBM) population selected with concentrations of esfenvalerate 5% EC (KOBII-esfenvalerate selected strain)[a], LC_{50} of DBM susceptible strain (KOBII-NS strain)[a] and upper limit of recommended field concentration

KOBII-esfenvalerate selected strain LC_{50} (mg/l) (95% CI)	*KOBII-NS strain LC_{50} (mg/l) (95% CI)*	*KOBII-esfenvalerate selected strain LC_{50} (mg/l) (95% CI)*	*Upper limit of recommended field concentration (mg/l)*	*Upper limit of recommended field concentration (mg/l)*	*KOBII-NS strain LC_{50} (mg/l) (95% CI)*	*Resistance ratio*
155 (99.4–206)[a]	0.698 (0.56–0.886)[a]	-	-	-	-	222[ab]
-	-	155 (99.4–206)[a]	50	-	-	3.1[c]
-	-	-	-	50	0.698 (0.56–0.886)[a]	71.6[d]

[a] Cited from Ninsin (2004a)

[b] Resistance ratio = LC_{50} KOBII-esfenvalerate selected strain ÷ LC_{50} KOBII-NS strain

[c] Resistance ratio = LC_{50} KOBII-esfenvalerate selected strain ÷ upper limit of recommended field concentration

[d] Resistance ratio = Upper limit of recommended field concentration ÷ LC_{50} KOBII-NS strain

Tables 1-4 show the RR computations using LC_{50} values of four strains of DBM from Japan, selected with phenthoate 50 per cent EC (Elsan®; organophosphate), cartap 50 per cent WP (Padan®; nereistoxin analogue), acetamiprid 20 per cent SP (Mospilan®; neonicotinoid) and esfenvalerate 5 per cent EC (Sumialpha®; pyrethroid) (Ninsin, 2004a), LC_{50} of a laboratory developed susceptible DBM strain from Japan (Ninsin, 2004a) and the upper limit of recommended field concentration for DBM control in Japan. In Japan, 1,000 – 1,500 dilution times of phenthoate 50 per cent EC and cartap 50 per cent WP and 1,000 – 2,000 dilution times of acetamiprid 20 per cent SP are the recommended field concentrations for DBM control. Esfenvalerate 5 per cent EC is not recommended for DBM control in Japan, but it is cited in this paper as a representative insecticide of the pyrethroids.

The upper limit of the recommended field concentration of insecticides for DBM control in Japan which is 1,000 dilution times is used for esfenvalerate 5 per cent EC. A consideration of Table 1 shows that the correct calculation of RR of KOBII-phenthoate selected strain yields 140-fold resistance, which indicates that the strain has high resistance to phenthoate. However, when the RR is calculated according to Odhiambo *et al.* (2010) and Botwe *et al.* (2012), the

KOBII-phenthoate selected strain yields only 1.22-fold resistance (Table 1), which suggests that the strain is susceptible to phenthoate. When the recommended field concentration of 500 mg/l is considered as the susceptibility of a field population or selected strain, an RR of 115 is obtained (Table 1), which indicates high phenthoate resistance.

The resistant KOBII-phenthoate selected strain is judged susceptible when its susceptibility is compared to the recommended field concentration because at susceptibility level equivalent to the field recommended concentration, a high frequency of phenthoate-resistance conferring genes exists, which causes the true RR of KOBII-phenthoate selected strain to be underestimated by a factor of 115 (Table 1). It is, therefore, conceivable that the RRs of the DBM populations reported by Odhiambo *et al*. (2010) and Botwe *et al*. (2012) have been seriously underestimated. Thus, field populations of DBM in Ghana are much more resistant to the insecticides tested than the reports indicate and would be rendering insecticides ineffective.

The goal of resistance management is to prevent or retard the development of resistance in field populations of arthropod pests, by continuously making available a high frequency of insecticide susceptible individuals in the pest population. Therefore, the detection of resistance in an early stage of development is essential to the success of resistance management (Ninsin *et al*., 2000; Antwi-Agyakwa *et al*., 2014) as it allows for the early implementation or tweaking of resistance management strategies to prevent or retard full-blown resistance to a particular insecticide or acaricide in an arthropod pest population. However, if the RR is calculated with the field recommended concentration according to Odhiambo *et al*. (2010) and Botwe *et al*. (2012), resistance to an insecticide or acaricide in its early stage of development would not be detected. This is because there would have been a significant accumulation of resistant individuals in the population and causing insecticides and acaricides to be ineffective before a decrease in the susceptibility of the population is detected.

Additionally, if the RR is calculated according to Odhiambo *et al*. (2010) and Botwe *et al*. (2012), and the susceptibility of a test population to insecticides or acaricides is determined to be equivalent to the recommended field concentration, i.e., RR=1, the population will be judged as completely susceptible, as declared for the University of Ghana DBM population tested against emamectin benzoate 1.9 per cent EC by Botwe *et al*. (2012). The recommendation for a test population with RR=1 would be that the insecticide or acaricide could still be used to control the susceptible pest population. Given that the genes that confer resistance to the insecticide or acaricide already abound in a pest population with susceptibility equivalent to the recommended field concentration, the continuing use of the products only causes further concentration of the resistance genes which results in a highly resistant population that renders the insecticides or acaricides completely ineffective.

Conclusion

The loss of insecticidal and acaricidal efficacy leads to control failure of field populations of arthropod pests which results in decreased agricultural productivity, increased incidence of arthropod-borne diseases, increased pesticide residues in foods and environmental contamination. It has been shown in this paper that when the RR of a field population of arthropod pest is calculated according to Odhiambo *et al*. (2010) and Botwe *et al*. (2012) with the field recommended concentration, the resistance level of the population is greatly underestimated so that even when the method detects an insignificant decrease in susceptibility, there is already a significant accumulation of resistance genes which causes insecticides and acaricides to become ineffective. It is, therefore, important to calculate the RR correctly with a susceptible strain which would indicate the true resistance level of a test population and permit the detection of resistance in an early stage of development to allow for the implementation or

tweaking of resistance management strategies to preserve the efficacy of insecticides and acaricides. In order to ensure that RRs from resistance research are reliable and useful in the management of insecticide and acaricide resistance development in arthropod pest populations, it is recommended that a national reference laboratory susceptible strain is established for each arthropod pest that has the genetic potential to develop resistance to insecticides or acaricides, so that the strain is used as the standard susceptible strain for resistance research in Ghana.

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