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Response of gut bacteria of chlorantraniliprole resistant and susceptible populations of diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) to antibiotics

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Abstract
The aim of this study was to evaluate the gut bacteria of chlorantraniliprole-resistant (CRD) and susceptible (CSD) populations of diamondback moth, *Plutella xylostella* susceptibility to different kinds of antibiotics. The gut bacteria were isolated from chlorantraniliprole-resistant (CRD) and –susceptible (CSD) populations of *P. xylostella* by simple serial dilution and plating method. Bacterial isolates from both populations were selected based on their colony morphology and tested for antibiotic susceptibility using ten different antibiotics by Kirby-Bauer disk diffusion method. Totally 12 and 13 bacteria were selected from chlorantraniliprole-resistant (CRD) and –susceptible (CSD) populations respectively. Most of the selected gut bacterial colonies were yellow to light pink in colour and were gram negative in reaction. All the bacterial isolates from CRD were sensitive to Cefpodoxime (CPD) and all the isolates were resistant to Cephallexin (CN) except isolate CDK 6 which was found sensitive. All the bacterial isolates from CSD showed sensitivity towards Gentamycin (G), Cefpodoxime (CPD) and Sulfafurazol (S) and almost all the isolates were found resistant to Rifampicin (R) except isolate NDKS 1 whose response was moderately sensitive. The results indicated that, the gut bacteria from both populations were sensitive to Cefpodoxime (CPD) and in overall, the gut bacteria from CRD were more resistant to antibiotics than CSD.

Keywords: diamondback moth, gut bacteria, antibiotics, cefpodoxime (CPD)

Introduction
Plutella xylostella, is the most serious pest in cruciferous crops such as cabbage, mustard and cauliflower *etc*. It causes huge loss in economically important crops [1]. It has developed resistance to almost all the chemical insecticides because of its short life cycle, capacity for rapid development of resistance, target mutations, gut microbes [2, 3] and some detoxifying enzymes such as cytochrome P450 monooxygenase, carboxylesterase and glutathion S transferase [4]**.** A novel diamide insecticide, Chlorantraniliprole has been widely used to control diamondback moth. High level of resistance to this insecticide was reported only after few years of its use in China [5], Philippines [6], and Brazil [7].

It is known that gut microbes of insects play an important role in the insect's metabolism, vitamin synthesis, pathogen prevention and detoxification. So, knowledge about these gut microbes is the key step to develop novel control strategies. Diamondback moth harbors many gut bacteria which are involved in detoxification of insecticides. The diversity of gut microbes in DBM, quantification of detoxifying enzymes (cytochrome P450 monooxygenase, carboxylesterase and glutathion S transferase) activity and elucidation of their possible role in degradation of indoxicarb were studied. Totally 25 bacterial isolates were obtained and result showed that *B. cereus* degraded indoxicarb upto 20 per cent [4]. The degradation of acephate, utilizing it as carbon and energy for the growth by the diamondback moth gut isolates *Bacillus cereus* (PXB. C.Or), *Enterobacter asburiae* (PXE), *Pantoae agglomerans* (PX-Pt.ag.Jor) provides a strong evidence that the bacterial communities present in the gut of diamondback moth might aid in acephate degradation and play a role in the development of insecticide resistance [8].

The best method to remove gut bacteria from insect is by the use antibiotics. The effect of five antibiotics (rifampicin, ampicillin, tetracycline, streptomycin sulfate and chloramphenicol) on the gut bacterial diversity of *P. xylostella* larvae were evaluated.

The results found that rifampicin and streptomycin sulfate at 3mg/mL significantly reduced the diversity of the bacterial community and tetracycline was observed to be most toxic [9]. The effect of five different concentrations of eight antibiotics on gut bacterial diversity of *P xylostella* were investigated by subjecting bacterial culture (ISO-1) from gut homogenates to antibiotic screening tests and by screening antibiotics against larvae of the insect where Cefixime (5 mg/ml) was found to be the most effective antibiotic with the greatest inhibition zone (25 mm). Higher mortality and reduced growth of larvae in case of larvae feeding on cefixime-treated leaves were recorded as compared to other treatments which suggested that bacterial symbionts play a crucial role in the successful development of the host [10].

In the present study, we aimed to test ten different antibiotics against gut bacteria of insecticide resistant and susceptible population of diamondback moth (*Plutella xylosetla*).

Materials and Methods

Isolation of gut bacteria from chlorantraniliprole-resistant (CRD) and –susceptible (CSD) population

Total of twenty number of third instar larvae of diamondback moth were selected from each population to isolate the gut bacteria and selected larvae were starved for 24 h. The starved larvae were surface sterilized with 70 per cent ethanol followed by 0.1 per cent NaOCl to remove disinfectant. The whole guts were dissected using dissection box and homogenization of gut was done with 0.1 M PBS (pH-7). The gut bacteria were isolated by serial dilution and plating method using nutrient agar (NA) and MacConkey agar. The plates were incubated for 24-48h at 30ºC. Colonies were selected based on size, shape, colour etc and pure culture was prepared for selected individual isolates by streak plate method. Gram staining was done to know the gram reaction of selected gut bacterial isolates.

Screening of antibiotics susceptibility of selected gut bacteria from CRD and CSD

The selected bacterial isolates from each population was tested for antibiotics susceptibility. Ten antibiotics *viz* Ampicilin (A/S) (10 mcg), Sulfafurazol (S) (30 mcg), Rifampicin (R) (5 mcg), Chloramphenicol (CHL) (10 mcg), Gentamycin (G) (10 mcg), Amoxycilin (AMX) (10 mcg), Cephallexin (CN) (30 mcg), Doxycyclin (DO) (30 mcg), Cefpodoxime (CPD) (10 mcg) and Tetracyclin (TET) (30 mcg) were used to screen the antibiotic sensitive bacterial isolates. Screening of antibiotics was carried out *in vitro* [11]**.** Spread plate method was done by adding 0.1 ml broth culture of all the isolates on NA medium and antibiotic discs were placed above the medium. The plates were incubated at 30ºC for 24 h. Inhibition zones were measured in diameter (Fig 1).

Results and Discussions

From chlorantraniliprole-resistant (CRD) and -susceptible (CSD) populations of DBM, a total of 12 and 13 gut bacterial isolates were selected respectively based on their morphology. The bacterial colonies selected from CRD were small, yellow to light pink in colour, seven isolates were gram negative and five isolates were gram positive in reaction. Similarly, from CSD, colonies were small, white to yellow in colour, 11 isolates were gram negative and remaining were gram positive in nature.

Almost all the gut bacterial isolates from CRD were resistant to Chloramphenicol (CN) except isolate CDK 6 which was CN sensitive, followed by Rifampicin (R) and sensitive to Cefpodoxime (CPD), followed by Gentamycin (G). Isolate CDK 6 was shown maximum inhibition zone by Tetracyclin (TET) (40.66 mm) (Table 1 and Fig. 2). Similarly, from CSD, all the isolates were resistant to Rifampicin (R) except isolate NDKS 1, followed by Cephallexin (CN) and all isolates were sensitive to Sulfafurazol (S), Gentamycin (G) and Cefpodoxime (CPD). Maximum inhibition zone was found in isolate NDKS 4 by Cefpodoxime (CPD) (Table 2 and Fig 3). The gut bacteria of *spodoptera litura* were eliminated by antibiotic administration to test insect larvae to study the mediated insecticide resistance. Bioassay was performed against lab reared and field collected larvae, results indicates that both lab and field population of test insects were more resistant in the presence of gut bacteria by recording higher LC50 values against test insecticides. In the absence of gut bacteria both lab and field collected larvae were comparatively susceptible which recorded lower LC50 values. Field larval population recorded 10.85, 17.00 and 53.39 ppm of LC50 for fluebendiamide, indoxocarb and chlorpyriphos respectively in the absence of gut bacteria. Similarly against lab reared antibiotic treated larval population test insecticides flubendiamide, indoxocarb and chlorpyriphos recorded LC50 values of 3.55, 7.96 and 20.22 ppm, respectively [12].

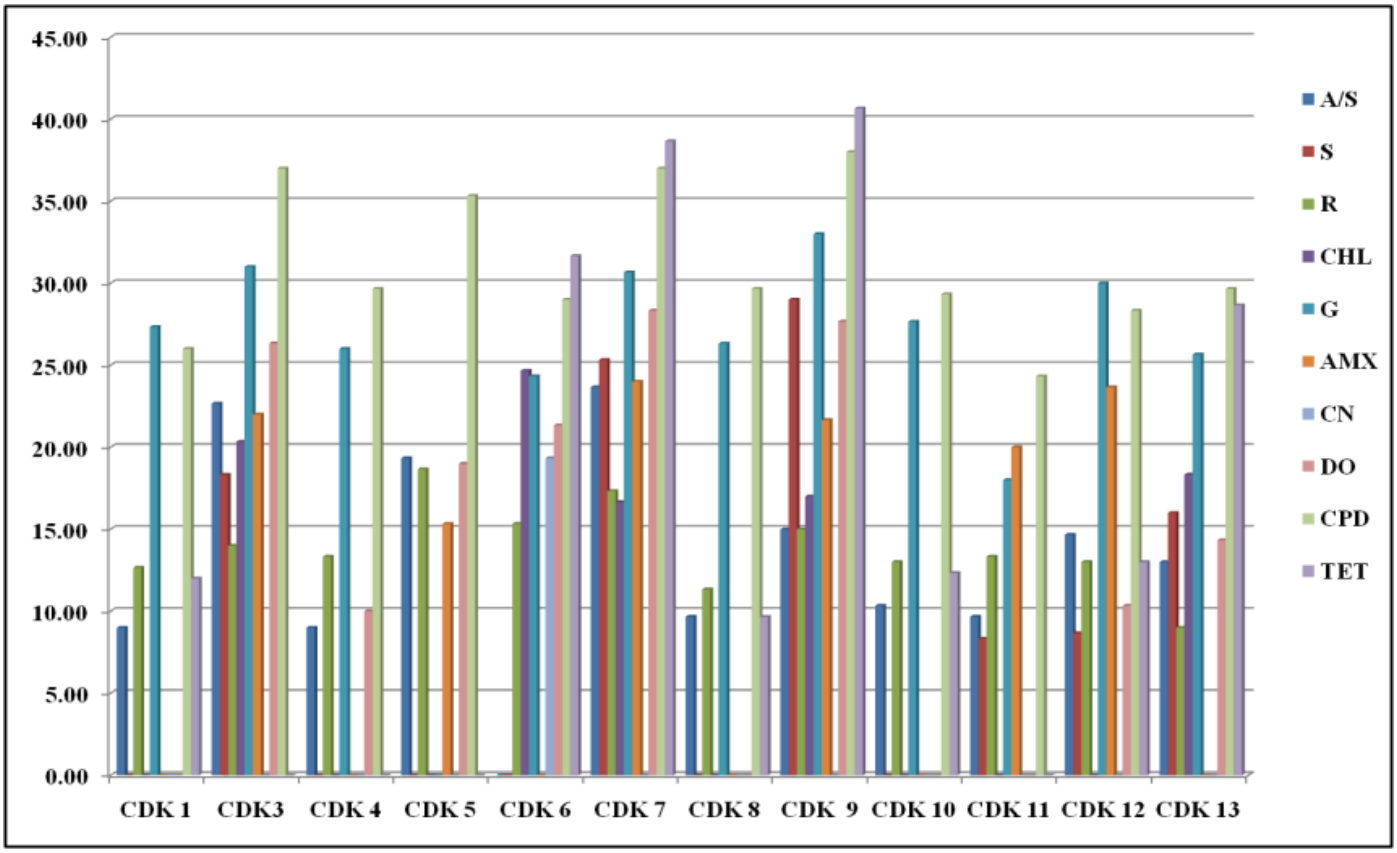
The effect of antibiotic rifampin on the gut microbial diversity, longevity, fecundity, and weight of two termite species, *Zootermopsis angusticollis* and *Reticulitermes flavipes* were described. The antibiotic rifampin causes a permanent reduction in the diversity of gut bacteria and a transitory effect on the density of the protozoan community, reduced colony fitness and the initial dosages of rifampin had severe long-term fitness effects on *Z. angusticollis*. A causal relationship between these changes in the gut microbial population structures and fitness was suggested by the acquisition of opportunistic pathogens and incompetence of the termites to restore a pretreatment, native microbiota. The results indicated that antibiotic treatment significantly alters the termite's microbiota, reproduction, colony growth and development [13].

The effects of gut bacteria and antibiotics on the fitness of Diamondback moth were studied. Two DBM strains such as reared radish DBM strain and the germ-free artificial diet DBM strain under antibiotics were taken and evaluated the effects of gut bacteria and antibiotics on the fitness of DBM. Results showed that the antibiotic treatment on the radish DBM strain has reduced host fitness, reflected as retarded development, reduced weights, declined pupation rates, descended fecundity, and shorted adult lifespan. The antibiotic treatment on the germ-free artificial diet DBM strain decreased pupation rate and fecundity. the negative effects on the host fitness after antibiotic treatment were partly caused by the toxic effect of antibiotic and partly by the deficiency of gut bacteria. The gut bacteria may play a promotive role in the fitness of DBM [14].

Table 1: Inhibition zone of gut bacteria from chlorantraniliprole resistant population of DBM by antibiotics (in mm)

Isolate code	A/S	S	R	CHL	G	AMX	CN	DO	CPD	TET
CDK 1	09.00(R)	0.00 (R)	12.66(R)	00.00(R)	27.33(S)	00.00(R)	00.00(R)	00.00(R)	26.00(S)	12.00(I)
CDK3	22.67(S)	18.33(S)	14.00(R)	20.33(S)	31.00(S)	22.00(S)	00.00(R)	26.33(S)	37.00(S)	00.00(R)
CDK 4	09.00(R)	0.00(R)	13.33(R)	00.00(R)	26.00(S)	00.00(R)	00.00(R)	10.00(R)	29.66(S)	00.00(R)
CDK 5	19.33(S)	0.00(R)	18.66(I)	00.00(R)	00.00(R)	15.33(I)	00.00(R)	19.00(S)	35.33(S)	00.00(R)
CDK 6	00.00(R)	0.00(R)	15.33(R)	24.67(S)	24.33(S)	00.00(R)	19.33(S)	21.33(S)	29.00(S)	31.66(S)
CDK 7	23.67(S)	25.33(S)	17.33(I)	16.67(I)	30.66(S)	24.00(S)	00.00(R)	28.33(S)	37.00(S)	38.66(S)
CDK 8	09.67(R)	0.00(R)	11.33(R)	00.00(R)	26.33(S)	00.00(R)	00.00(R)	00.00(R)	29.66(S)	09.66(R)
CDK 9	15.00(I)	29.00(S)	15.00(R)	17.00(I)	33.00(S)	21.66(S)	00.00(R)	27.67(S)	38.00(S)	40.66(S)
CDK 10	10.33(R)	0.00(R)	13.00(R)	00.00(R)	27.66(S)	00.00(R)	00.00(R)	00.00(R)	29.33(S)	12.33(I)
CDK 11	09.67(R)	08.33(R)	13.33(R)	00.00(R)	18.00(S)	20.00(S)	00.00(R)	00.00(R)	24.33(S)	00.00(R)
CDK 12	14.67(I)	08.66(R)	13.00(R)	00.00(R)	30.00(S)	23.66(S)	00.00(R)	10.33(R)	28.33(S)	13.00(I)
CDK 13	13.00(R)	16.00(I)	09.00(R)	18.33(S)	25.66(S)	00.00(R)	00.00(R)	14.33(R)	29.66(S)	28.66(S)

* Ampicilin (A/S), Sulfafurazol (S), Rifampicin (R), Chloramphenicol (CHL), Gentamycin (G), Amoxycilin (AMX), Cephallexin (CN), Doxycyclin (DO), Cefpodoxime (CPD) and Tetracyclin (TET). *R- Resistant, *S- Sensitive



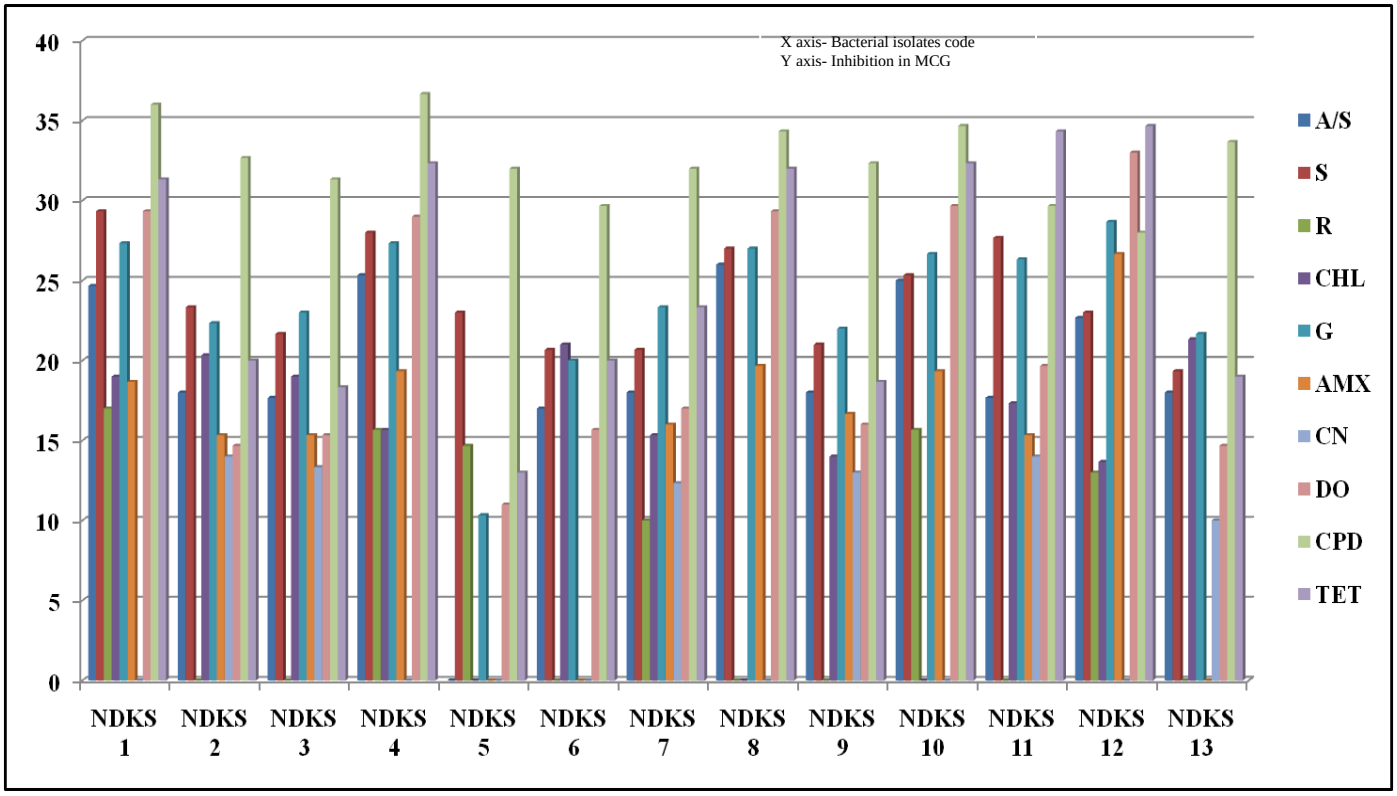
***** Ampicilin (A/S), Sulfafurazol (S), Rifampicin (R), Chloramphenicol (CHL), Gentamycin (G), Amoxycilin (AMX), Cephallexin (CN), Doxycyclin (DO), Cefpodoxime (CPD) and Tetracyclin (TET)

Fig 2: Sensitivity of gut bacteria isolates from chlorantraniliprole resistant DBM by selected antibiotics.

Table 1: Inhibition zone of gut bacteria from chlorantraniliprole-susceptible population of DBM by antibiotics (in mm)

Isolate code	A/S	S	R	CHL	G	AMX	CN	DO	CPD	TET
NDKS 1	24.66 (S)	29.33(S)	17.00(I)	19.00(S)	27.33(S)	18.66(S)	00.00(R)	29.33(S)	36.00(S)	31.33(S)
NDKS 2	18.00(S)	23.33(S)	00.00(R)	20.33(S)	22.33(S)	15.33(I)	14.00(S)	14.67(S)	32.66(S)	20.00(S)
NDKS 3	17.67(S)	21.66(S)	00.00(R)	19.00(S)	23.00(S)	15.33(I)	13.33(R)	15.33(S)	31.33(S)	18.33(S)
NDKS 4	25.33(S)	28.00(S)	15.66(R)	15.67(I)	27.33(S)	19.33(S)	00.00(R)	29.00(S)	36.66(S)	32.33(S)
NDKS 5	00.00(R)	23.00(S)	14.66(R)	00.00(R)	10.33(S)	00.00(R)	00.00(R)	11.00(I)	32.00(S)	13.00(I)
NDKS 6	17.00(S)	20.66(S)	00.00(R)	21.00(S)	20.00(S)	00.00(R)	00.00(R)	15.67(S)	29.66(S)	20.00(S)
NDKS 7	18.00(S)	20.66(S)	10.00(R)	15.33(I)	23.33(S)	16.00(I)	12.33(R)	17.00(S)	32.00(S)	23.33(S)
NDKS 8	26.00(S)	27.00(S)	00.00(R)	00.00(S)	27.00(S)	19.66(S)	00.00(R)	29.33(S)	34.33(S)	32.00(S)
NDKS 9	18.00(S)	21.00(S)	00.00(R)	14.00(I)	22.00(S)	16.66(I)	13.00(R)	16.00(S)	32.33(S)	18.67(S)
NDKS 10	25.00(S)	25.33(S)	15.66(R)	00.00(R)	26.66(S)	19.33(S)	00.00(R)	29.67(S)	34.66(S)	32.33(S)
NDKS 11	17.67(S)	27.66(S)	00.00(R)	17.33(I)	26.33(S)	15.33(I)	14.00(S)	19.67(S)	29.66(S)	34.33(S)
NDKS 12	22.67(S)	23.00(S)	13.00(R)	13.67(I)	28.66(S)	26.66(S)	00.00(R)	33.00(S)	28.00(S)	34.66(S)
NDKS 13	18.00(S)	19.33(S)	00.00(R)	21.33(S)	21.66(S)	00.00(R)	10.00(R)	14.67(S)	33.66(S)	19.00(S)

* Ampicilin (A/S), Sulfafurazol (S), Rifampicin (R), Chloramphenicol (CHL), Gentamycin (G), Amoxycilin (AMX), Cephallexin (CN), Doxycyclin (DO), Cefpodoxime (CPD) and Tetracyclin (TET). *R-Resistant, *S- Sensitive



***** Ampicilin (A/S), Sulfafurazol (S), Rifampicin (R), Chloramphenicol (CHL), Gentamycin (G), Amoxycilin (AMX), Cephallexin (CN), Doxycyclin (DO), Cefpodoxime (CPD) and Tetracyclin (TET)

Fig 2: Sensitivity of gut bacteria isolates from chlorantraniliprole susceptible DBM by the selected antibiotics

Conclusion

The study revealed that, the gut bacteria from both CRD and CSD populations were very sensitive to Cefpodoxime. Gut bacterial isolates from CRD were resistant to Chloramphenicol (CN) and from CSD were resistant to Rifampicin (R). Over all, the gut bacteria of chlorantraniliprole-resistant populations (CRD) were more resistant to selected antibiotics than chlorantraniliprole susceptible populations (CSD) of *P. xylostella*. Further investigations are needed to study the effect of antibiotics which gave the best results against the larvae of both CRD and CSD populations for the management of *P. xylostella* in combination with chlorantraniliprole.

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