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Research Note

Effect of combinations of *Vairimorpha necatrix* (Kramer) with antibiotics on the susceptibility of *Spodoptera litura* (Fabricius)

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ABSTRACT: ***Vairimorpha necatrix*** **(Kramer) was combined with different antibiotics to increase its pathogenicity. It was administered to third instar larvae of** ***Spodoptera litura*** **(Fabricius) under laboratory conditions by diet surface treatment and the mortality and spore yield was studied. Reproductive spores of** ***V. necatrix*** **at 10^6 spores/ml were mixed with 100 ppm of antibiotics** ***viz.*** **gentamycin, oxytetracycline, ampicillin, kanamycin and chloramphenicol. The results showed that the larval mortality due to various** ***Vairimorpha*** **antibiotic combinations ranged from 10 to 62.07 per cent and it was high compared to pre-pupal and pupal stages. Assessment of spore yield in the infected larvae, pre-pupae and pupae revealed that larval stages recorded higher production of spores which ranged from 0.23 to 2.36 x 10^{10} spores for larva, 2.26 to 5.28 x 10^9 spores for pre pupa and 0.28 to 2.23 x 10^9 spores for pupa. Among the various combinations,** ***Vairimorpha*****-gentamycin combination produced higher mortality and spore yield of** ***V. necatrix.***

KEY WORDS: Antibiotics, mortality, *Spodoptera litura*, spore yield, *Vairimorpha necatrix*

Vairimorpha necatrix (Kramer) is one among the several microorganisms used in the control of crop pests of economic importance. These pathogens produce infections of nature that range from sub-acute and chronic to acute and virulent but these are considered to be less effective when compared to that of insect pathogenic viruses (Nordin and Maddox, 1972; Fuxa, 1979). *V. necatrix* can be mass-produced on live hosts (Fuxa and Brooks, 1979) and has provided good control in some field experiments (Jaques, 1977). Maximum production of spores is essential for the effective mass production of the pathogen for use in the integrated pest management. In the present investigation, the effect of combination of different antibiotics on the mortality and spore yield in *Spodoptera litura* (Fabricus) were studied.

An isolate of *V. necatrix* was isolated from tobacco cutworm, *S. litura* collected during a field survey from castor fields of Tiruchirappalli during winter 1998. The infected larvae were typically pinkish in colour with abnormally large fat bodies. Wet mounts of tissue smears under phase contrast microscopy revealed the presence of spores of the pathogen. The spores recovered were administered to the homologous host in the laboratory at a concentration of 3.9 x 10^3 spores/third instar larvae for obtaining sufficient inoculum of the pathogen. A diet surface treatment method (Hunter and Hall,

1968) was used in bioassays adopted (Maddox and Solter, 1996). At death, the larvae were collected in 1.5 ml eppendorf tubes singly and utilized for the study. The spores from larvae were recovered by differential centrifugation (Undeen and Alger, 1971) and the strength (spores/ml) of the suspension was assessed.

A laboratory bioassay was conducted to study the effect of certain antibiotics when combined with *V. necatrix* on the mortality and spore yield of *S. litura*. *V. necatrix* spores at a concentration of 10^6 spores/ml (counted using haemocytometer) was suspended in 100 ppm of different antibiotics, *viz.* gentamycin, oxytetracycline, ampicillin, kanamycin and chloramphenicol. *V. necatrix* alone at 10^6 spores/ml was maintained as control. A casein based semi-synthetic diet (Shorey and Hale, 1965) of *S. litura* (lacking formaldehyde) was prepared and dispensed in 5 ml glass vial. 10 ml of the suspension was applied on the surface of semi-synthetic diet and distributed evenly with the help of glass rod. Each treatment was replicated four times with 25 third instar larvae/replication in individual vials. Third instar *S. litura* larvae each weighing 50 mg was released into each glass vial and kept at room temperature of 25°C. When the larvae exhausted the diet, fresh set of untreated semi-synthetic diet of 30 mg was provided. The observation on the mortality of the insect were recorded at 24 hours intervals until pupation. The cadavers in different treatments were collected @ five per replication in separate eppendorf tubes and the spore yield was assessed. The individual samples were ground in all glass pestle and mortar and the volume of the homogenate was made upto 10 ml with distilled water. The spore concentrationin unpurified suspensions were enumerated with the help of haemocytometer. (Neubauer)

The results of the bioassay showed that among the different *Vairimorpha* antibiotic combinations tested, *Vairimorpha* + gentamycin combination was found to be highly effective by resulting in total mortality of 85.58 per cent followed by *Vairimorpha* alone which recorded 65.96 per cent mortality. The larval mortality was found to be higher (62.07%) compared to pre-pupal (11.82%) and pupal mortality (11.69%). The highest larval mortality of 62.07 per cent was obtained by exposing the third instar larvae to *V. necatrix* spore suspension at 10^6 spores/ml suspended in gentamycin 100 ppm (Table 1). All the other

Table 1. Effect of combinations of *V. necatrix* with antibiotics on the mortality of *Spodoptera litura*

Treatment *	Per cent mortality in different stages**			Total mortality (%)
	Larvae	Pre pupae	Pupae	
Vairimorpha + gentamycin	62.07^a	11.82^a	11.69^a	85.58^a
Vairimorpha + oxytetracycline	10.67^e	4.52^d	9.00^d	24.19^e
Vairimorpha + ampicillin	32.14^c	9.41^b	10.71^b	52.26^c
Vairimorpha + kanamycin	18.79^d	7.82^c	10.71^b	37.32^d
Vairimorpha + chloramphenicol	10.00^e	4.27^d	9.72^c	23.99e
Vairimorpha alone	47.14^b	9.11^{bc}	9.71^c	65.96^d
SEM±	2.42	0.06	0.05	2.56
CD (P=0.05)	6.80	1.14	0.10	7.10

* *V. necatrix* @ 10^6 spores/ml; Antibiotics @ 100 ppm

**Means followed by similar letters are not statistically significant by DMRT (P=0.05).

Table 2. Production of spores in *V. necatrix* antibiotic combinations at different stages after inoculation

Treatment *	Spore yield in different stages**		
	Larva x 10^{10}	Pre-pupa x 10^{9}	Pupa x 10^{9}
Vairimorpha + gentamycin	2.36^{a}	5.28^{a}	2.23^{a}
Vairimorpha + oxytetracycline	1.09^{cd}	2.48^{c}	1.17^{b}
Vairimorpha + ampicillin	1.18^{c}	2.37^{cd}	0.97^{c}
Vairimorpha + kanamycin	1.06^{d}	2.27^{d}	0.28^{e}
Vairimorpha + chloramphenicol	1.04^{d}	2.26^{d}	0.78^{d}
Vairimorpha alone	1.58^{b}	4.02^{b}	2.13^{a}
SEM±	0.03	0.04	0.07
CD (0.05)	0.08	0.13	0.18

**V. necatrix* @ 10^{6} spores/ml; Antibiotics @ 100 ppm

**Means followed by similar letters are not statistically significant by DMRT (P=0.05)

antibiotic combinations produced significantly lesser larval mortality compared to *Vairimorpha* alone. The *Vairimorpha* gentamycin combination recorded the highest pre-pupal mortality followed by *Vairimorpha* ampicillin and *Vairimorpha* alone. No significant difference was observed between the *Vairimorpha* antibiotic combinations in the pupal stage.

Observations recorded on the spore yield from infected insects revealed that it ranged from 0.28 x 10^{9} to 2.36 x 10^{10} spores for the various stages analyzed. The spore yield was highest in the larval stage followed by pre pupal and pupal stages, which were on par (Table 2). The spore yield/larva and pre-pupae were highest in the *Vairimorpha* gentamycin combination, which recorded 2.36 x 10^{10} spores/larva and 5.28 x 10^{9} spores/pre pupa. All the other combinations recorded significantly lesser larval and prepupal spore yield compared to *Vairimorpha* alone.

The *Vairimorpha* gentamycin and *Vairimorpha* alone were on par with each other with respect to spore yield/pupa. The present findings are in confirmation with Kurinjiselvan, (1994) who reported that higher yield of the microsporidian was obtained when *Vairimorpha* 10^{6} spores/ml was suspended in Neomycin 100ppm. Pilley (1978) found that the antibiotics, tetracycline, neomycin and kanamycin were effective in suppressing bacterial growth in spore suspensions of *V. necatrix*. The antibiotics could have eliminated the gut microflora and exposed the surface area of the gut for initial attack by *V. necatrix* alone.

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