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EVALUATION OF CERTAIN BOTANICALS, BIOAGENTS AND INSECTICIDAL FORMULATIONS AGAINST THE GRUBS OF COCONUT RHINOCEROS BEETLE (*Oryctes rhinoceros* L. SCARABAEIDAE: COLEOPTERA)

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ABSTRACT

Studies were conducted to know the efficacy of certain biopesticides and bioagents against grubs of coconut rhinoceros beetle, *Oryctes rhinoceros* L. under laboratory conditions. Biopesticides - Neem seed kernel powder (NSKP), Pongamia seed kernel powder (PSKP); Entomopathogenic nematodes (EPN) - *Heterorhabditis indica*, *H. bacteriophora*; Entomopathogenic fungi (EPF) - *Metarhizium anisopliae*, *Beauveria bassiana* and chlorantraniliprole 0.4% G were evaluated. EPNs - *H. indica* and *H. bacteriophora* were recorded highly effective and 46.67 and 33.33% mortality, respectively was observed at 5 days after treatment (DAT) and 100% kill was observed by 10 DAT. In EPFs - *Metarhizium anisopliae* and *Beauveria bassiana* treatments cent percent mortality was observed by 15 and 20 DAT, respectively. Treatment with Chlorantraniliprole 0.4% G recorded complete mortality of grubs by 20 DAT. NSKP and PSKP recorded 100% mortality of grubs by 25 and 30 DAT, respectively. Our studies indicate the potential of entomopathogenic nematodes, entomopathogenic fungi and botanicals for the management of *O. rhinoceros* grubs.

Key words : Coconut rhinoceros beetle, *Oryctes rhinoceros*, Entomopathogenic nematodes, Entomopathogenic fungi, Botanical insecticides, Biopesticides, Biological control.

Introduction

Coconut rhinoceros beetle, *Oryctes rhinoceros* L. is an important pest of coconut palms in tropical regions including Andhra Pradesh (Chalapathirao *et al*., 2018). Adults bore into unopened fronds causing characteristic V-shaped cuts, resulting in reduced photosynthetic area, stunting, yield loss and even mortality of young palms (Srinivasan *et al*., 2018). Rhinoceros beetle grubs (larvae) feed on decaying organic matter like rotting coconut wood, fallen palms and compost. Abundant breeding media for adult beetles such as decomposing organic matter, farmyard manure pits, sawdust heaps and palm residues favour its persistence and spread (Pille and Ceniza, 2018). Management of larval stages by using botanicals, bioagents has been studied in view of environmental safety.

In the present study, 2 botanicals (Neem Seed Kernel Powder, Pongamia Seed Kernel Powder), 2 entomopathogenic fungi (*Metarhizium anisopliae*, *Beauveria bassiana*) and 2 entomopathogenic nematodes (*Heterorhabditis indica, H. bacteriophora*) and Chlorantraniliprole 0.4% G (farmer's practice) were evaluated against larvae of *O. rhinoceros* under laboratory conditions.

Materials and Methods

Botanicals (Neem Seed Kernel Powder, Pongamia Seed Kernel Powder) were prepared using standard method (Anonymous, 2017). Entomopathogenic fungal cultures, *Metarhizium anisopliae* and *Beauveria bassiana* used for the experiments were obtained from

CPCRI, Kasaragod and HRS, Ambajipeta, respectively. Entomopathogenic nematodes (EPNs), *Heterorhabditis indica* and *H. bacteriophora* infected insect cadavers of *Galeria melonella* were obtained from NIPHM, Hyderabad. These treatments were tested against 2nd instar larvae of *O. rhinoceros* under laboratory conditions in the Biocontrol laboratory, Dr. YSRHU- Horticulture Research Station, Ambajipeta.

Culturing of *O. rhinoceros*

To start this experiment, a total of 240 *O. rhinoceros* grubs were collected from the breeding sites, such as manure pits and decaying logs, in coconut plantations around Ambajipeta (Fig. 1). The collected grubs were reared in FYM filled plastic containers in the laboratory. From these grubs, second instars were selected based on their size and weight were used for the study. Throughout the study, laboratory temperature was maintained at 26 ± 2°C and relative humidity of 70 ± 5%. The moisture content of the FYM was also maintained at an optimum level by periodically sprinkling of water to prevent desiccation. The trays were kept in a well-ventilated laboratory environment, avoiding direct sunlight to ensure favourable conditions for normal grub activity.

Table 1 : List of botanicals, bioagents and insecticides evaluated against rhinoceros beetle grubs.

S. no.	Treatments	Dosage
T_1	Neem Seed Kernel Powder	50 g/ m^2
T_2	Pongamia Seed Kernel Powder	50 g/ m^2
T_3	*Heterorhabditis indica*	150 juveniles/ pot or 1 cadaver/ pot
T_4	*H. bacteriophora*	150 juveniles/ pot or 1 cadaver/ pot
T_5	*Metarhizium anisopliae* (1×10^8 spores/ mg)	10 g/ m^2
T_6	*Beauveria bassiana* (1×10^8 spores/ mg)	10 g/ m^2
T_7	Chlorantraniliprole 0.4% G	1.5 g/ m^2
T_8	Control	Untreated



Fig. 1 : Collection of grubs from field.

Test procedure

Eight treatments, each replicated three times, were evaluated using plastic tubs (45 cm radius) containing 3 kg of well-decomposed farmyard manure (FYM) and mixed with selected treatments. Powder formulations of neem seed kernel powder, pongamia seed kernel powder, *Beauveria bassiana* and *Metarhizium anisopliae*, along with chlorantraniliprole granules, were thoroughly mixed with the FYM to ensure uniform distribution. The aqueous suspension containing extracted infective juveniles (IJs) of entomopathogenic nematodes was sprinkled onto the FYM and mixed uniformly. 10 numbers of second instar grubs were released in each tray and the trays were covered with cotton cloth and secured with rubber bands to prevent the escape of grubs while allowing adequate aeration. Observations were done 5 days interval up to 30 days after treatment.

Mortality (%)

$$Per\ cent\ Mortality = \frac{Number\ of\ dead\ grubs\ observed}{Total\ number\ of\ grubs} \times 100$$

(Srinivasan *et al.*, 2018)

Statistical analysis

Analysis was done with ANOVA under completely randomised block design and the mean values were compared by following Duncan's multiple range test (DMRT) with a P value of 0.05. (Gomez and Gomez, 1984). Before the analysis, the necessary data trans formations were performed and the calculations were done by using the computer-based OPSTAT package was used for the calculation.

Results and Discussion

In terms of percent larval mortality, significant variations were observed across the eight treatments (Table 1). Among the treatments, the nematode formulations, *Heterorhabditis indica* and *H. bacteriophora*, exhibited the fastest insecticidal activity, against second instar grubs of *O. rhinoceros*, causing 46.67% and 33.33% mortality, respectively, at 5 days after treatment (DAT) and 100% mortality was achieved by 10 DAT. The infected cadavers, when observed under microscope showed the presence of nematodes (Fig. 2). These results are consistent with Patil *et al.* (2014), who evaluated *H. indica* against *O. rhinoceros* grubs and reported that mortality varied with nematode species and exposure duration. Similarly, Sujithra *et al.* (2022) reported

Table 2 : Effect of different biopesticides and insecticidal formulation on mortality of grubs of rhinoceros beetle, *O. rhinoceros*.

S. no.	Treatment detail	Cumulative Percent Mortality (%)					
		5 DAT	10 DAT	15 DAT	20 DAT	25 DAT	30 DAT
1	Neem Seed Kernel Powder	0.00±0.00^{d} (1.25)	13.33±2.67^{de} (17.69)	46.67±5.41^{b} (41.8)	80.00±0.00^{b} (60.82)	100.00±0.00^{a} (77.45)	100.00±0.00^{a} (77.45)
2	Pongamia Seed Kernel Powder	6.67±5.59^{cd} (9.47)	13.33±3.18^{de} (17.69)	53.33±4.63^{b} (47.17)	73.33±3.09^{b} (58.57)	93.33±6.55^{a} (71.91)	100.00±0.00^{a} (77.45)
3	*Heterorhabditis indica*	46.67±2.37^{a} (41.8)	100.00±0.00^{a} (77.45)	100.00±0.00^{a} (77.45)	100.00±0.00^{a} (77.45)	100.00±0.00^{a} (77.45)	100.00±0.00^{a} (77.45)
4	*Heterorhabditis bacteriophora*	33.33±4.11^{ab} (34.06)	100.00±0.00^{a} (77.45)	100.00±0.00^{a} (77.45)	100.00±0.00^{a} (77.45)	100.00±0.00^{a} (77.45)	100.00±0.00^{a} (77.45)
5	*Metarhizium anisopliae* (1×10^{8} spores)	26.67±1.98^{b} (29.98)	66.67±7.11^{b} (53.03)	100.00±0.00^{a} (77.45)	100.00±0.00^{a} (77.45)	100.00±0.00^{a} (77.45)	100.00±0.00^{a} (77.45)
6	*Beauveria bassiana* (1×10^{8} spores)	26.67±3.29^{b} (29.98)	53.33±3.09^{bc} (45.7)	93.33±6.55^{a} (71.91)	100.00±0.00^{a} (77.45)	100.00±0.00^{a} (77.45)	100.00±0.00^{a} (77.45)
7	Chlorantraniliprole 0.4% G	20.00±2.60^{bc} (21.76)	33.33±3.55^{cd} (29.5)	60.00±5.00^{b} (49.36)	100.00±0.00^{a} (77.45)	100.00±0.00^{a} (77.45)	100.00±0.00^{a} (77.45)
8	Control	0.00±0.00^{d} (1.25)	0.00±0.00^{e} (1.25)	0.00±0.00^{c} (1.25)	0.00±0.00^{c} (1.25)	0.00±0.00^{b} (1.25)	0.00±0.00^{b} (1.25)
	SE(m)	0.67	1.72	0.98	0.71	0.29	0.00
	C.D (p= 0.05)	1.99	4.18	2.98	2.45	1.08	0.00

* Mean of 3 replications Values in parenthesis are arc transformed Values with various alphabets differ significantly.

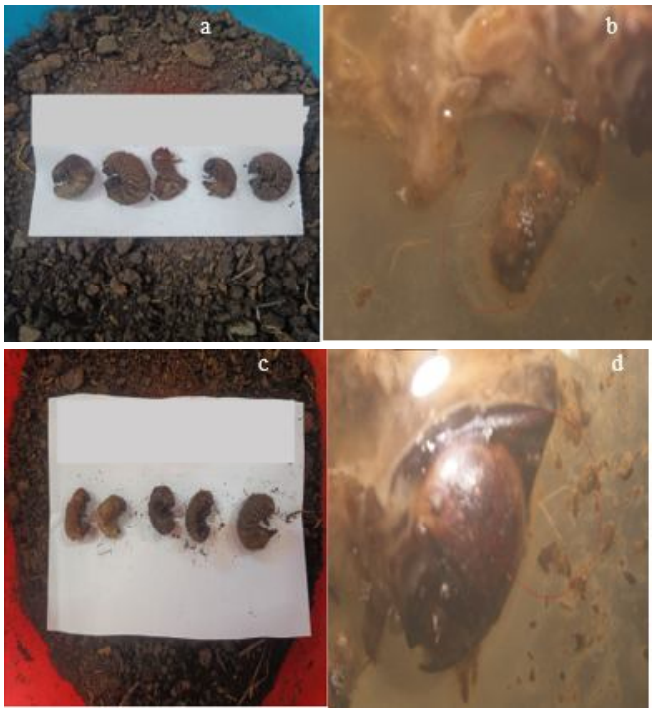


Fig. 2 : a. Grubs infested EPN with *H. indica* and b. microscopic observation of the presence of nematode, c. Grubs infested EPN with *H. bacteriophora* and d. microscopic observation of the presence of nematode.

that the virulence of native EPN strains against second-instar *O. rhinoceros* larvae was influenced by nematode concentration and exposure period.

The rapid mortality caused by EPNs may be associated with the ability of infective juveniles to locate and penetrate the host and release their symbiotic bacteria, which contribute to insect death. Ishibashi and Kondo (1990); Peters and Ehlers (1994) discussed the importance of host-finding and penetration in EPN infection, while Epsky and Capinera (1993) examined factors influencing nematode host finding and infection. These mechanisms provide a possible explanation for the rapid and complete mortality observed in the *H. indica* and *H. bacteriophora* treatments.

The entomopathogenic fungi, *Metarhizium anisopliae* caused 66.67% mortality at 10 DAT and complete mortality was recorded in 15 DAT, whereas *Beauveria bassiana* recorded 53.33% mortality at 10 DAT and 93.33% at 15 DAT, reaching 100% mortality by 20 DAT. These findings are supported by Khudhair *et al.* (2015), who evaluated *M. anisopliae* and *B. bassiana* against larvae of the Arabian rhinoceros beetle, *Oryctes agamemnon arabicus* and reported that both fungi caused high mortality, reaching 100% after 29 days under their experimental conditions. Similarly, the relatively faster and higher mortality caused by *M. anisopliae* in the present study agrees with the findings of Saleem and Ibrahim (2019) demonstrated the pathogenicity of both

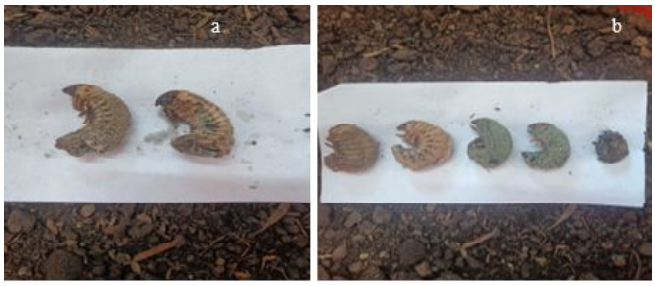


Fig. 3 : EPF infected grubs showing fungal mycelial growth a. *Beauveria bassiana*, b. *Metarhizium anisopliae*.



Fig. 4 : Botanicals treated grubs of rhinoceros beetle turned black and hardened.

fungal species reporting maximum mortality of 60.8% with *M. anisopliae* and 55.5% with *B. bassiana*. The sporulation of fungi covering the full body of the insect was 5.40 ± 0.14 days in *M. anisopliae* treatment and 6.40 ± 0.14 days in treatment *B. bassiana*, suggesting a faster growth of *M. anisopliae* (Fig. 3).

Among the botanicals, neem seed kernel powder (NSKP) and pongamia seed kernel powder (PSKP) produced slower but progressive mortality. At 15 DAT, PSKP recorded 53.33% mortality compared with 46.67% for NSKP. However, NSKP achieved complete mortality by 25 DAT, whereas PSKP reached complete mortality by 30 DAT. The dead grubs were observed by reduction of size, blackening and hardened (Fig. 4).

Chlorantraniliprole 0.4% G caused 33.33% mortality at 10 DAT, increasing to 60.00% at 15 DAT and reaching complete mortality by 20 DAT. Complete mortality was recorded in all treated groups at 30 DAT, whereas no mortality occurred in the untreated control.

Conclusion

Overall, the findings indicate that EPNs (*Heterorhabditis indica*, *H. bacteriophora*) provided rapid grub mortality, followed by fungal formulations (*Metarhizium anisopliae*, *Beauveria bassiana*) and botanicals (Neem Seed Kernel Powder, Pongamia Seed Kernel Powder). chlorantraniliprole achieved complete mortality over a longer period. The study highlights the efficiency of EPNs, EPFs and botanicals for the management of *O. rhinoceros* grubs.

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