

<https://doi.org/10.48047/AFJBS.6.15.2024.3440-3447>



African Journal of Biological Sciences

Journal homepage: <http://www.afjbs.com>



Research Paper

Open Access

## Effect of frequent exposures of Mancozeb on *Rhynocoris fuscipes* (Hemiptera: Reduviidae) a natural enemy of groundnut, *Arachis hypogaea* Linn. Insect pests.

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Volume 6, Issue 15, 2024

Received: 15 May 2024

Accepted: 18 Aug 2024

Published: 05 Sep 2024

[doi:10.48047/AFJBS.6.15.2024.3440-3447](https://doi.org/10.48047/AFJBS.6.15.2024.3440-3447)

**Abstract:** This study investigates the toxicity of the fungicide mancozeb on predatory bug *Rhynocoris fuscipes*. Female *R. fuscipes* were collected from groundnut plants in Pepeganj and reared in the laboratory for mancozeb exposure experiments. The bugs were subjected to the field- recommended concentration of mancozeb at varying frequencies (one dip, weekly dip, daily dip, and no dip) to assess mortality rates, developmental duration, and prey consumption. Exposure to mancozeb resulted in either an increase ( $14.3 \pm 0.5$  days) or decrease ( $4.3 \pm 0.5$  days) in nymphal duration, a reduction in prey consumption capacity ( $2 \pm 0$ ), and elevated mortality rates (60%, 66.66%, 100%) at third, fourth, and fifth nymphal stages in the daily dip (DD) category. Additionally, mancozeb significantly impacted prey consumption capacity ( $5.8 \pm 0.6$ ,  $5.9 \pm 0.3$ ), developmental duration ( $48.8 \pm 0.2$ ,  $46.9 \pm 0.2$  days), and mortality percentages (30.2%, 40%, and 16.66%, 33.33%) at third and fourth nymphal stages in the weekly dip (WD), and one dip (OD) categories respectively.

**Key words:** Groundnut field, Fungicide effect, beneficial bug, *Rhynocoris fuscipes*, Prey consumption, Developmental duration, Mortality rate,

### Introduction

Groundnut, or peanut, *Arachis hypogaea* Linn. Ranks as the second most significant grain legume crop globally, following soybean (USDA, 2006). A major threat to its yield is the fungal leaf spot disease, caused by

*Cercospora arachidicola* and *Cercosporidium personatum*, which severely impacts susceptible varieties (Mohammed et al., 2018). More than 100 insect pest species infest groundnut, with notable ones including larvae of *Spodoptera litura*, and *Helicoverpa armigera*, which feed gregariously on leaves and flower buds. Adults and nymph stages of *Aphis craccivora* also pose significant threats by sucking sap from tender shoots and flowers (Nataraj et al., 2014). *Rhynocoris fuscipes* is a critical predator of various insect pests and a vital biocontrol agent, contributing significantly to pest suppression in groundnut fields (Ambrose 1999; Sahayaraj 1999; Sahayaraj and Martin 2003). Another beneficial reduviid, *R. marginatus* has been effectively used to manage pest populations in groundnut crops (Sahayaraj, 1999). *R. fuscipes* preys on a wide range of pests, including larvae of *Spodoptera litura*, *Helicoverpa armigera*, and *Aphis craccivora* (Personal observation). Mancozeb, an ethylene bis dithiocarbamate fungicide, is widely used to control various fungal pathogens viz., *Phytophthora* sp., *Alternaria* sp., *Cercospora* sp., and *Botrytis* sp., to protect groundnut from fungal leaf spot disease (Adamski et al., 2011). Despite its efficacy, mancozeb is relatively benign to the predatory mite *Amblyseius andersoni* concerning female mortality rates (Ioriatti et al., 1992). However, it has been shown to increase larval periods and mortality of *S. litura* (Srivastava et al., 2017), and to cause reduced survivability, disturbance, and malformations in larvae and imago of *S. exiuga* (Adamski and Ziemnicki, 2004). These detrimental effects extend to non-target species, highlighting the need for careful application and monitoring.

## Material and Methods

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- a) **Site of Experiment:** The study was conducted at the Entomology unit of the Zoology Department, in St. Andrew's College, Gorakhpur, and Uttar Pradesh.
- b) **Collection and rearing of reduviids:** Fecund females of *Rhynocoris fuscipes* were collected during evening hours (6:00 PM to 9:00 PM) in July 2023 from the ventral leaf surfaces of groundnut plants at Mahayogi Gorakhnath Krishi Vigyan Kendra Chaukmafi (Lati: 26.93012° N, 83.23686° E) Peppeganj by using mobile torch. The collected insects were reared with head-crushed grasshoppers, *Hieroglyphus banian*, in plastic containers (20cm diameter, 6cm in height) supplemented with dry, green leaves to mimic natural environmental conditions. A wet cotton bud was included to maintain humidity. Laboratory conditions were maintained at 24±1°C to 27±1°C temperature, 70% - 80% RH, and a Photoperiod of 13L: 11D hours. A stock culture of reduviid nymphs was established under these conditions.
- c) **Fungicide toxicity studies:** Mancozeb, obtained from a local agricultural pesticide supplier, was dissolved in tap water to prepare a stock fungicide solution at their field recommended concentration (75% WP). Twenty four hours old fresh and healthy first nymphal instars were used for this experiment. Initially a fresh paired leaf of groundnut is dipped inside fungicide stock solution with the help of forceps. After 10 second, this fungicide dip leaf (replaced after 24 hours) is kept in a plastic container (9.5cm diameter, 4.5cm height) for experiment and without dipped leaf kept for control. After that immediately twenty four hours old first nymphal instars of *R. fuscipes* was introduced into container with jumping legs removed and head crushed grasshopper provided as source of nutrition. This fungicide dipped leaf experiment was in three categories namely daily, weekly, and one dip in each categories. Eighteen replicates were maintained.
- d) **Toxicity Assay:** The impact of chronic mancozeb exposure on prey consumption, developmental duration, and mortality in *R. fuscipes* was assessed. Control experiments used water-treated leaves to

account for experimental errors. Observations continued until adult emergence, with longevity record in DD, WD, OD, and control ND (control) categories. Data were analyzed for mortality rates corrected for fungicide treatment by using Abbott's formula (1925).

## Result and Discussion

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The chronic toxicity effect of fungicide, mancozeb on prey consumption capacity, developmental duration, and mortality of *R. fuscipes* were mentioned in tables 1, 2 and figures 1, 2. The results indicated that mancozeb treatment led to a marked decrease in prey consumption capacity and prolonged developmental duration across all nymphal stages (fig.2).

**1) Number of prey consumption:** The bug eats less when exposed to the fungicide. The mortality rates were also notably higher in the treated groups compared to the controls. The feeding capacity of *R. fuscipes* was significantly reduced ( $<1 \pm 0$ ) at the third nymphal stage and completely lost at the fourth nymphal stage in DD category. Prey consumption capacity of *R. fuscipes* was also reduced in WD, and OD categories compared to control. The average number of prey consumed in DD, WD, OD, and ND categories was  $2 \pm 0.0$ ,  $5.8 \pm 0.6$ ,  $5.9 \pm 0.3$ , and  $6.3 \pm 0.1$ , respectively. This study underscores the anti-feeding effect of fungicide on non-targeted beneficial arthropod (Chalfant et al 1977; Sahayaraj et al., 2003). Fungicide, Euparen M was very effective in the reducing the feeding of *S. littoralis* (Aziz et al., 2002). The fungicide, azoxyastrobin and tebuconazole reduced feeding ratio and reduced the average weight of consumed larvae, pupation (%), pupal mortality (%), pupal duration (days) and adult emergence (Ramadan et al., 2018).

**2) Developmental duration:** The time takes for the bug to reach adulthood increases. This fungicide, mancozeb exhibited a deleterious impact on the developmental duration, survival of the nymph of reduviid, *R. fuscipes* (Table 1). Mancozeb greatly altered the normal development by either increased or decreased nymphal duration. The highest ( $14.3 \pm 0.5$  days) and lowest ( $4.3 \pm 0.5$  days) stadial period was recorded at third and fourth nymphal stages in DD, as compared to WD, OD, and control categories. It was also increases mortality at third, fourth, and fifth nymphs and furthermore adult did not emerged out in DD category. These results align with previous studies highlighting the negative impact of fungicide on the developmental of reduviid. Mancozeb increased the larval period and mortality of *S. litura* (Srivastava et al., 2017). Among the tested three treatment categories, two treatment categories (WD, and OD) showed a significantly prolongation of nymphal developmental duration compared to control. The mortality percentage also reduced in WD, and OD categories. Exposure of *A. Segetum* larvae to mancozeb has produced greater effect on mortality and duration of larval development (Adamski et al., 2011). Fungicide, Ridomil MZ affects the growth and development of *S. litura* (Srivastava et al., 2017).

The nymphal developmental duration of *R. fuscipes* in WD, OD, and control categories were  $48.8 \pm 0.2$ ,  $46.9 \pm 0.2$ ,  $46.6 \pm 0.2$  days whereas adult longevity were  $35.6 \pm 0.8$ ,  $37.8 \pm 0.8$ , and  $39.3 \pm 0.7$  days.

Mancozeb (75WP) increased the larval and developmental periods in *S. litura* (Singh and Bhattacharya 2004).

**3) Mortality:** More bugs dies when exposed to the fungicide. The percentage of mortality was calculated in both “treatment” as well as “control” categories to estimate accurate results. Fungicide, mancozeb did not cause any mortality that is 0% in first and second nymphal instars of *R. fuscipes*. Carbendazim, a fungicide, did not have significant effects on larval and pupal mortality in *Bombyx mori* (Bizhannia et al., 2005). Fungicide, mancozeb causes 60%, 66.66%, and 100% mortality at third, fourth, and fifth nymphal stages in DD category, 30.2%, 40%, 0% and 0% mortality at third, fourth, fifth, and adult stages in the WD. Mortality rates were 16.66%, 33.33%, 0% and 0% at the third, fourth, fifth, and adult stages in OD category, respectively. This finding underscores the potential risks associated with the indiscriminate use of mancozeb, particularly its adverse mortality rate on *R. fuscipes*. Fungicide, tebuconazole exhibits high insecticidal efficacy by disrupting microbial homeostasis in *Nilaparvata lugens* (Cai et al., 2023). Fungicide, mancozeb caused no mortality in first and second nymphal stages at low concentration, but the percentage of mortality gradually increased as nymphs grew older in the presence of fungicide due to high concentration of the chronic exposure of *R. fuscipes*. However, exposure of a predatory mite *Typhlodromus pyri* to residues of mancozeb did not affect egg hatch, fecundity, developmental rate or mortality in the field (Baynon and Penman., 1987). Mancozeb is a contact fungicide with low toxicity to non-target species, but continuous exposure can be harmful (Yousuf et al., 2023). Auger et al., (2004) stated that the susceptibility of the predatory mite *T. pyri* population to the mancozeb fungicide was reduced, and female survival, fecundity and the viability of female progeny were affected by mancozeb in laboratory.

Table 1- Impact of exposure frequency of mancozeb on duration of nymphal development (DND) and number of prey consumed (NPC) of predator, *R. fuscipes*.

Life Stage	Mancozeb exposure frequency							
	One dip (OD)		Weekly dip (WD)		Daily dip (DD)		No dip (ND)	
	DND	NPC	DND	NPC	DND	NPC	DND	NPC
I	$9.3 \pm 0.7a$	$1.0 \pm 0.0$	$9.5 \pm 1.0a$	$1.0 \pm 0.0$	$10.3 \pm 0.1a$	$1.0 \pm 0.0$	$9.3 \pm 0.9a$	$1.0 \pm 0.0$
II	$7.5 \pm 0.1ab$	$1.0 \pm 0.0$	$8.3 \pm 0.5b$	$1.0 \pm 0.0$	$4.6 \pm 1.0a$	$1.0 \pm 0.0$	$7.3 \pm 1.0ab$	$1.0 \pm 0.0$
III	$7.6 \pm 0.5a$	$1.0 \pm 0.0$	$8.6 \pm 0.5ab$	$1.0 \pm 0.0$	$14.3 \pm 0.5b$	$1.0 \pm 0.0$	$7.6 \pm 0.5a$	$1.0 \pm 0.0$

iv	11.4±0.5b	1.4±0.5	11.2±0.5b	1.1±0.3	4.3±0.5	N.F.	11.3±0.5b	1.5±0.6
v	11.1±0.9a	1.5±0.6	11.2±0.9a	1.7±0.7	Blank	Blank	11.1±1.0a	1.8±0.3
Total stadia	46.9±0.2a	5.9±0.3	48.8±0.2a	5.8±0.6	33.5±0.2a	2.0±0.0	46.6±0.2a	6.3±0.1
Adult longevity	37.8±0.8a	2.4±0.5	35.6±0.8a	2.42±0.5	Blank	Blank	39.3±0.7a	2.57±0.5

Mean value followed by different alphabets are statistically significant (DMRT).

Table 2- Fungicide, mancozeb on the corrected % mortality of predator, *R. fuscipes* (DD= Daily Dip, WD= Weekly Dip, OD= One Dip, and ND= No Dip).

Life Stage	OD	WD	DD
I	0%	0%	0%
II	0%	0%	0%
III	16.66%	30.2%	60%
IV	33.33%	40%	66.66%
V	0%	0%	100%
Adult	0%	0%	Blank



Figure 1- Alive nymph in “control “categories, b- Dead nymph in “Treatment” categories.

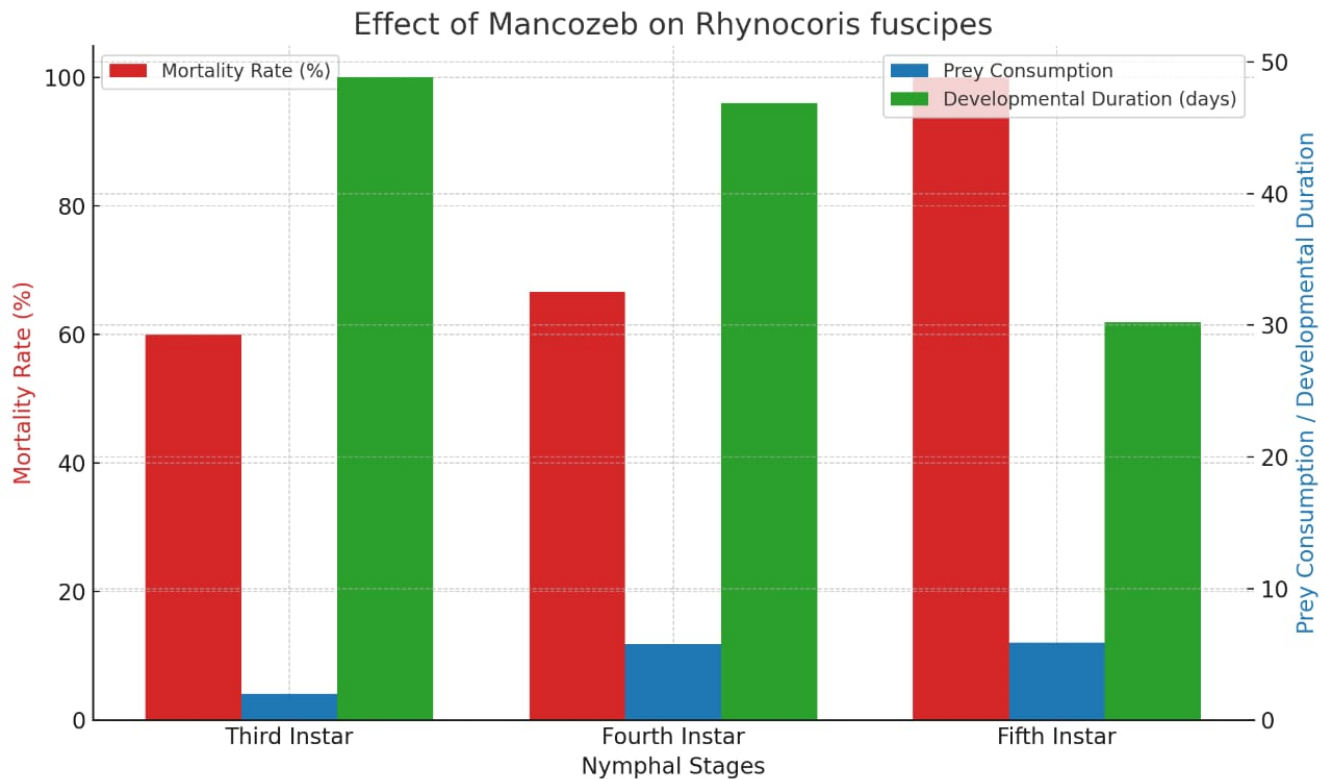


Figure 2- Effect of Mancozeb on mortality rate, prey consumption, and developmental duration across the 3<sup>rd</sup>, 4<sup>th</sup>, and 5<sup>th</sup> nymphal instars of *Rhynocoris fuscipes*.

## Conclusion

- 1) *R. fuscipes* is a predatory bug that feeds on various insect pests in groundnut field, including *Spodoptera litura*, *Helicoverpa armigera*, and *Aphis craccivora*. Mancozeb is a fungicide used to control fungal disease in groundnut crops, but it can also have negative effects on beneficial organisms like *R. fuscipes*.
- 2) The fungicide may decremented the sensory cells of legs, antennae, and rostrum of *R. fuscipes*. Sothat reduced food consumption is recorded. The legs are not in proper shape, curved in certain cases, and reduced mobility also observed. Moreover, the abnormal molting is the main cause of death, so that it may suggest that it alter the hormone in balance.
- 3) The study suggested that continuous application of fungicide in agro-ecosystem causes environmental issue and may interfere with normal physiological and biochemical process of non-target species of agro-ecosystem.
- 4) Hence, applications of fungicide reduced the population, increased mortality and disrupt normal nymphal development of this predatory bug. This could lead to an increase in pest problems as the bugs would not be there to control them naturally.
- 5) Further research should explain alternative fungicide formulation and application methods that mitigate the adverse effect on non-target species while effectively controlling pathogenic fungi.

**Acknowledgements:**

The authors are thankful to Prof. CO Samuel, Principal and Secretary, St. Andrew's College for providing laboratory facilities and encouragement. The junior author is acknowledged to his Ph.D. advisory committee members Prof. Veena Batra Kushwaha and Prof. Keshav Singh for their great concern and encouragements.

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