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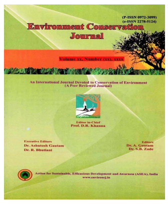

Synergism of inert minerals and botanicals with spinosad: Co-toxicity against *Callosobruchus maculatus* in stored blackgram

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ABSTRACT

The investigation of "Inert Minerals and Botanicals Synergism with Spinosad: co-toxicity against *Callosobruchus maculatus* in Stored Blackgram" was performed in the Laboratory of the Department of Entomology, Agricultural College, Bapatla, during 2020-21. The inert minerals and botanicals were mixed with Spinosad and used for the bioassay. The data were recorded at 24, 48 and 72 hours after the release of the test insect, and probit analysis was performed to calculate the LD_{50}. By using the LD_{50} of inert minerals and botanicals alone and in combination with spinosad, the co-toxicity coefficient was calculated. Among all the treatments, the highest co-toxicity coefficient (433.33) was recorded for diatomaceous earth, indicating increased efficacy and synergism between diatomaceous earth and Spinosad.

Introduction

Blackgram or urdbean is an important pulse crop with its origin in India, where it is the largest producer, consumer, and importer globally. With a protein content of approximately 26%, nearly three times that of cereals, it plays a vital role in the daily diet of the vegetarian population in India (Directorate of Marketing and Inspection, Ministry of Agriculture and Farmers Welfare, GOI, 2020). However, the crop faces a significant threat from bruchids, particularly *Callosobruchus maculatus* (Fabricius), a widespread and destructive pest that penetrates mature pods both in the field and during postharvest storage (Nahdy, 1995). The harm inflicted by this pest results from egg laying on grain surfaces followed by larval penetration into the grains. This attack leads to reduced grain weight, diminished retail value, compromised quality and nutritional content, decreased product hygiene, and lower seed germination rates (Faroni and Sousa, 2006). Due to its short life cycle and high reproductive capacity, losses can reach up to 100% within six months of storing untreated produce (Mkenda *et al*., 2014).

Numerous synthetic insecticides have demonstrated efficacy against stored-product insect pests; however, their use has posed significant risks to humans and domestic animals. Over the past four decades, the excessive and indiscriminate application of synthetic pesticides, particularly insecticides, has resulted in a range of issues, such as insecticide resistance, pest resurgence, residues in both food and soil, and environmental pollution (Mohapatra and Gupta, 1998). To address these challenges, scientists have explored alternative pest management approaches, focusing on solutions derived from plant sources (Islam *et al*., 2013). According to Subramanyam & Roesli (2000), inert dusts exert a physical impact on insects, inducing abrasions on their bodies and resulting in the breakdown of the lipid layer on the external surface

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of the cuticle, ultimately leading to insect death due to dehydration. In recent years, spinosad, a biopesticide derived from the naturally occurring soil actinomycete *Saccharopolyspora spinosa*, has gained increased amounts of attention. Recognized for its limited nontarget and chronic effects, it was designated a "reduced risk" compound by the EPA (Environmental Protection Agency) in 1993. Subsequently, in 2005, the EPA registered Spinosad as a grain protectant at a concentration of 1 milligram per kilogram of active ingredient. Despite the main advantages of inert minerals and botanicals, they affect the physical and mechanical properties of grains (Losic and Korunic, 2018). To overcome these limitations, there is a need to integrate these substances with insecticides to enhance their effectiveness at lower concentrations. The primary objective of this study was to assess the efficacy of spinosad as a grain protectant when combined with inert minerals and botanicals against *C. maculatus*.

Material and Methods

The experiment was performed in the Entomology Department's laboratory at Agricultural College in Bapatla during 2020-21. *C. maculatus* cultures were acquired from the Post Harvest Technology Centre, Agricultural College, Bapatla. Minerals and botanicals such as diatomaceous earth (DE), China clay, paddy husk ash, cow dung ash, sweet flag powder, neem kernel powder and Spinosad 45 SC (suspension concentrate) were used. A bioassay was conducted on one-day-old adult *C. maculatus* beetles via the jute cloth disc impregnation method (Satyasri *et al.*, 2017). For this purpose, a two-milliliter aliquot of the insecticidal solution was used to fully impregnate nine-centimeter diameter jute cloth discs, which were subsequently air-dried and placed on Petri plates. Later, different doses of test inert minerals and botanicals were weighed and dusted on jute cloth discs placed in Petri plates. One-day-old beetles, collected from the culture, were starved for two hours and then transferred onto the treated jute cloth discs in petri plates, with 20 beetles per plate. The combined doses of minerals and botanicals with Spinosad are given in Table 1. The data were recorded 24, 48, and 72 h after the release of the test insect. The mortality percentage was corrected by using Abbott's formula (Abbott, 1925), and probit analysis was performed according to Finney (1971) using SPSS 20 (Statistical Package for Social Sciences) software to calculate the LD_{50} (median lethal dose) and other parameters for inert minerals and botanicals in combination with Spinosad. By using the LD_{50} of inert minerals and botanicals alone and in combination with Spinosad, the co-toxicity coefficient with respect to minerals and botanicals was calculated by using the following formula (Sun and Johnson, 1960):

$$\textbf{Co toxicity coefficient} = \frac{\textbf{LD}_{50}\textbf{ of the toxicant alone}}{\textbf{LD}_{50}\textbf{ of the toxicant in the mixture}} \times 100$$

When the co-toxicity coefficient of a mixture is 100, the effect of this mixture indicates the probability of similar action. A mixture with a coefficient significantly greater than 100 indicates synergistic action. Independent action usually results in a coefficient less than 100, but the toxicity of the mixture will be greater than that of either component.

Table 1: Doses of effective minerals and botanicals combined with spinosad according to the jute cloth disc method

S N	Treatments	Combined dosages (g/9 cm (d) jute cloth disc)
1.	Diatomaceous earth + Spinosad	0.00025+0.0025, 0.0005+0.005, 0.001+0.01, 0.002+0.02, 0.003+0.03, 0.004+0.04, 0.005+0.05
2.	China clay + Spinosad	0.0025+0.025, 0.005+0.05, 0.01+0.1, 0.02+0.2, 0.03+0.3
3.	Paddy husk ash + Spinosad	0.00125+0.0125, 0.0025+0.025, 0.005+0.05, 0.01+0.1, 0.02+0.2
4.	Cow dung ash + Spinosad	0.00125+0.0125, 0.0025+0.025, 0.005+0.05, 0.01+0.1, 0.02+0.2, 0.03+0.3
5.	Sweet flag powder + Spinosad	0.0005+0.005, 0.001+0.01, 0.002+0.02, 0.004+0.04, 0.006+0.06, 0.008+0.08, 0.01+0.1
6.	Neem kernel powder + Spinosad	0.0005+0.005, 0.001+0.01, 0.003+0.03, 0.005+0.05, 0.01+0.1, 0.02+0.2

Results and Discussion

The data on the co-toxicity coefficient values of inert minerals and botanicals from the jute cloth disc method against *C. maculatus* revealed that the highest co-toxicity coefficient value was observed for diatomaceous earth (433.33) at a combined LD_{50} value of 0.18 mg/cm^2 (Table 2), followed by sweet flag powder (403.22) (Table 3) at 3 days after

Table 2: Co-toxicity coefficient of inert minerals in combination with Spinosad

SN	Treatments	Combined dosages (g/9 cm (d) jute cloth disc)	Mortality (%)	LD_{50} of treatment alone (mg/cm^2)	LD_{50} of the treatment in combination with spinosad (mg/cm^2)	$\chi 2$	Slope b (±SE)	Regression Equation Y = a + bx	Co-toxicity Coefficient
1	Diatomaceous earth	0.00025 + 0.0025	16.66	0.78	0.18 (0.15-0.21)	6.59	1.67 (±0.12)	Y = 3.22 + 1.67X	433.33
		0.0005 + 0.005	36.66						
		0.001 + 0.01	50.00						
		0.002 + 0.02	60.00						
		0.003 + 0.03	70.00						
		0.004 + 0.04	80.00						
		0.005 + 0.05	90.00						
2	China clay	0.0025 + 0.025	13.33	4.87	1.96 (1.64-2.38)	3.73	1.54 (±0.16)	Y = 1.39 + 1.54X	248.46
		0.005 + 0.05	33.33						
		0.01 + 0.1	50.00						
		0.02 + 0.2	60.00						
		0.03 + 0.3	70.00						
3	Paddy husk ash	0.00125 + 0.0125	16.66	2.35	0.67 (0.56-0.81)	4.33	1.56 (0.15)	Y = 2.12 + 1.56X	361.53
		0.0025 + 0.025	36.66						
		0.005 + 0.05	56.66						
		0.01 + 0.1	76.66						
		0.02 + 0.2	80.00						
4	Cow dung ash	0.00125 + 0.0125	13.33	2.82	0.86 (0.73-1.00)	2.69	1.73 (±0.13)	Y = 2.18 + 1.73X	327.90
		0.0025 + 0.025	33.33						
		0.005 + 0.05	50.00						
		0.01 + 0.1	70.00						
		0.02 + 0.2	80.00						
		0.03 + 0.3	90.00						

Table 3: Co-toxicity coefficient of botanicals in combination with Spinosad

SN	Treatments	Combined dosages (g/9 cm (d) jute cloth disc)	Mortality (%)	LD_{50} of treatment alone (mg/cm^2)	LD_{50} of the treatment in combination with spinosad (mg/cm^2)	χ^2	Slope b (±SE)	Regression Equation Y = a + bx	Co-toxicity Coefficient
1	Sweet flag powder	0.0005 + 0.005	26.66	1.25	0.31 (0.25-0.36)	7.59	1.36 (±0.11)	Y = 2.33 + 1.36X	403.22
		0.001 + 0.01	43.33						
		0.002 + 0.02	50.00						
		0.004 + 0.04	63.33						
		0.006 + 0.06	70.00						
		0.008 + 0.08	80.00						
		0.01 + 0.1	90.00						
2	Neem kernel powder	0.0005 + 0.005	16.66	1.85	0.47 (0.31-0.72)	9.30	1.40 (±0.11)	Y = 2.12 + 1.40X	393.61
		0.001 + 0.01	26.66						
		0.003 + 0.03	36.66						
		0.005 + 0.05	66.66						
		0.01 + 0.1	76.66						
		0.02 + 0.2	90.00						

treatment. For all the treatments, the co-toxicity values were greater than 100, which indicated that there was a synergistic effect between spinosad and inert minerals and botanicals (Sun, 1950). All the inert minerals and botanicals tested were found to be more effective in combination with Spinosad, which is evident from the lower LD_{50} values of the combination treatment than of the treatments alone. Even at lower dosages, inert minerals and botanicals can cause greater mortality when combined with spinosad. All the treatments showed better independent effects at higher doses and good combined effects with lower doses of spinosad. The lower doses of combined dust and spinosad had similar effects on higher doses of dust and spinosad applied alone. The increased efficacy of very low doses of DE in combination with Spinosad may be due to the increased effect of Spinosad, which may be due to enhanced contact toxicity resulting from the abrasion of insect cuticular layers by DE (Korunic, 1998), which might have resulted in greater effects of spinosyn A and D on postsynaptic nicotinic acetylcholine receptors and gamma-aminobutyric acid (GABA) receptors in *C. maculatus*. These results pertaining to the combined effect of DE and Spinosad are in agreement with those of Korunic and Rozman (2010), who stated that the probable cause of the synergism between DE and deltamethrin is the combination of different modes of action, such as physical (desiccation) and chemical (toxicity) modes. Insects desiccated from DE and became weaker and less resistant to the toxic action of deltamethrin. Therefore, lower concentrations of deltamethrin in combination with DE are needed for the control of *Sitophilus zeamais*, *Rhyzopertha dominica*, and *Tribolium castaneum*. In the case of botanicals *viz.*, sweet flag powder and neem kernel powder also enhanced toxicity, which can be attributed to their predisposing effects on the test insect. Sweet flag powder contains active biological compounds such as *beta-asarones,* which penetrate the insect body through the cuticle and target acetylcholinesterase (AchE) and paralyze the insect (Melani *et al.*, 2016), followed by the addition of spinosad. Similarly, in the case of neem kernel powder, the principle bioactive compound azadirachtin, due to its biological effects, such as repellency and growth disruption (Doharey and Singh, 1989), might have weakened the insects more and led to an enhanced effect of combination treatment. Similarly, the results are in agreement with those of Ajiboye *et al.* (2023), who reported that admixing botanical powders, such as those from citrus species, with spintor is effective at protecting millet grains against infestations by *T. castaneum*. The mode of action of powder formulations that kill target arthropods involves the abrasion of the cuticle, which causes desiccation, and the blockage of the spiracle by dust particles (EPA, 1997).

Conclusion

The findings of this research reveal that when inert minerals and botanicals are combined, the highest co-toxicity coefficient occurs with diatomaceous earth and spinosad, suggesting significant synergism. This synergy increases fatality rates, enabling the use of lower doses. Consequently, the use of lower doses does not impact the physical attributes of stored grain, such as bulk density and flowability.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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