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Research article

NEEM FORMULATIONS – SAFER SEED PROTECTANTS AGAINST PULSE BEETLE, *CALLOSOBRUCHUS CHINENSIS* FOR LONG TERM STORAGE OF BENGALGRAM.

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ABSTRACT: The efficacy of neem products like neem oil and commercially available neem formulations *viz.,* Econeem plus®, Neemindia ® and Neemazal ® were evaluated in the laboratory for the control of pulse beetle, *C. chinensis* in stored bengalgram. These were compared with deltamethrin treatment as a chemical check. The bengalgram seed was treated with the neem formulations and stored under ambient conditions for storability studies. Observations on oviposition, insect damage, germination and seedling vigor index were recorded at three months interval. All the neem formulations were found to be effective against *C. chinensis* in stored bengalgram up to 12 months of storage. Surprisingly, deltamethrin treatment recorded highest oviposition of pulse beetle and insect damage resulted in poor germination and vigour of stored bengalgram seed. This gives an indication of resistance development in *C. chinensis* against commonly used synthetic pyrethroid insecticide deltamethrin in bengalgram. Crude neem oil @ 5 ml/ kg seed affected badly the germinability and seedling vigour of bengalgram seed under storage. The results indicated that the neem formulations viz., Neemazal, Econeem plus and Neemindia were found to be very effective against *C. chinensis* in stored bengalgram and also maintained high viability and vigour of seed up to twelve months of storage. Our results suggest that these neem formulations can be used as safer alternatives to chemicals for long term storage of pulses.

Key words: Bengalgram, *Callosobruchus chinensis,* Neem formulations, germination, Seedling vigor, Storage pests

INTRODUCTION

Bengalgram (*Cicer arietinum* L.) is an important food legume with good source of protein for human consumption. India is the world's largest producer of chickpea and the year 2009-10 witnessed a marked significant increase in chickpea area (8.25 m ha) recording a production of 74, 80,000 m tones.(FAOSTAT, 2010) In Andhra Pradesh also the increase in chickpea area is quite dramatic viz., 71,000 ha in 1991-93 to 5, 84,000 ha in 2010- 11 recording a productivity of 625 kg/ha in 1991-93 to 1233 kg/ ha in 2010-11. Significant losses in pulses, both quantitative and qualitative, occur during storage and storing pulses in storehouses to keep them free from being damaged by insect pests is a major problem. Amongst the stored grain pests of pulses, the pulse beetle, *Callosobruchus chinensis* L., is the economic pest (Ahmed *et al*., 2003). Gujar and Yadav (1978) reported 55-60% losses in seed weight and 45.50-66.30% losses in protein content due to its damage and the post harvest seed losses may reach up to 100% (Mahendran and Mohan, 2002) and the seeds become unfit for human consumption as well as planting.

In the recent past, the preservation of pulses has relied heavily upon the insecticides to control the storage pests. However, the present trend is towards alternative non-toxic control methods that pose no threat to the health of operator or consumer, and which are environmentally friendly. It is demanding to develop the alternative methods that are economically feasible and ecologically safer to control the storage grain insects (Moreno-Martinez *et al.,* 2000).

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The use of botanical pesticides is considered to be an alternative substitute to hazardous chemicals. Among the botanicals, Neem is visualized as an eco-friendly pesticide having rich source of bioactive chemicals with a greater potential for development as successful pest control agent which can affect insects in several ways: they may disrupt major metabolic pathways and cause rapid death, act as attractants, deterrents, phago-stimulants, anti-feedants or ovipositional deterrents, also retard or accelerate development or interfere with the life cycle of the insects. Hence, the present study was conducted to evaluate the efficacy of neem formulations on the pulse beetle, *Callosobruchus chinensis infesting* bengalgram and its effect on the long term storability and quality of bengalgram seeds.

MATERIALS AND METHODS

The laboratory and storage studies were conducted at Seed Entomology Laboratory, Seed Research and Technology Centre, Acharya N. G. Ranga Agricultural University, Rajendranagar, Hyderabad Andhra Pradesh. Freshly harvested, insect free and clean bengalgram variety 'KAK-2' obtained from Breeder seed production unit of S.R.T.C, Rajendranagar, Hyderabad was used for experimental purpose. The moisture content of bengalgram seed as determined by a Dickey John Moisture meter as 10%. Three commercially available neem formulations *viz.,* Econeem plus (Margo bio-controls private ltd., Bangalore, India), Neemindia (ITC Ltd., India) and Neemazal (EID Parry India Ltd, India) along with crude neem oil and chemical check deltamethrin were evaluated against pulse beetle in bengalgram seed. One kg of freshly harvested certified seed with high germination (> 90%) and low moisture content (10%) was used for experimentation. Required quantity of neem formulation was added and mixed thoroughly for proper coating on the seed. The seeds were packed in gunny bags of 2 kg capacity and kept in laboratory under ambient conditions of 27 ± 5^0 C temperature and 65 ±5% RH for natural infestation. The data recording was initiated after three months of storage and continued up to 12 months of storage at three months interval. Observations on oviposition and damage by pulse beetle, seed germination, and seedling vigor index were recorded at different storage intervals Germination test using paper towel technique was carried out as per the procedure given by ISTA (1999). The data were statistically analyzed using MSTAT package after suitable transformations.

RESULTS AND DISCUSSION

Effect of neem formulations on oviposition of pulse beetle

The effectiveness of different neem formulations on the oviposition of *C.chinensis* on bengalgram is expressed in Table 1 and Fig.1. At three months after storage, neem oil found to be superior without any egg laying on treated seeds followed by other neem formulations (20- 100 eggs/100 seeds) compared to chemical check deltamethrin and untreated control(60 eggs/100 seeds). Later, during six, nine and twelve months after treatment , there was no egg laying recorded with the bengalgram seed treated with neem formulations where as deltamethrin treatment recorded highest egg laying on the treated seeds (100, 860, 987, respectively) which is more than the untreated control. Hence, The cumulative mean number of eggs laid was more in the deltamethrin treatment (491.8) than in untreated control (157.5 eggs/100 seeds). The lowest number of eggs were deposited with Neemazal at both the test doses of 0.75 ml and 1.5 ml/kg seed (5 and 7.5 eggs/100 seeds respectively).

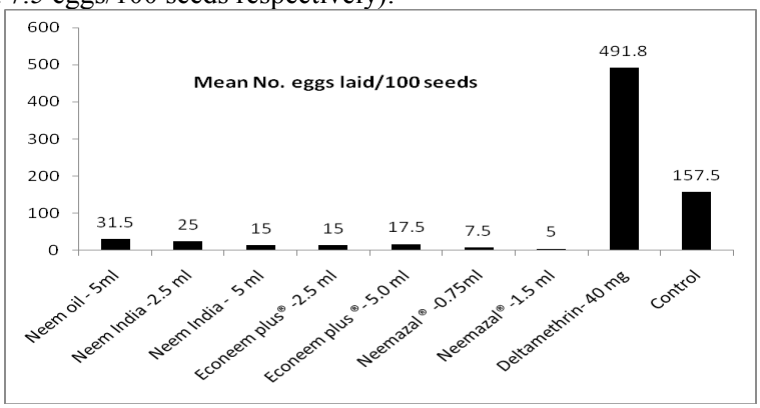

Fig 1. Effect of neem formulations on the oviposition of *C.chinensis* in bengalgram

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Table 1. Effect of neem formulations on the oviposition of pulse beetle and damage in bengalgram

Treatments (ml/kg seed)	No. eggs laid/100 seeds ** (months after storage)					Insect damage (%) * (months after storage)				
	3	6	9	12	Mean	3	6	9	12	Mean
Neem oil - 5ml	0 (0.71)	0 (0.71)	60 (7.5)	66 (7.5)	31.5 (5.6)	0 (0.71)	0.6 (4.4)	3.6 (10.6)	9.2 (17.6)	4.5 (12.0)
Neem India -2.5 ml	100 (10.0)	0 (0.71)	0 (0.71)	0 (0.71)	25.0 (4.9)	0 (0.71)	2.2 (8.5)	2.2 (8.5)	4.5 (12.0)	3.0 (9.5)
Neem India - 5 ml	60 (7.5)	0 (0.71)	0 (0.71)	0 (0.71)	15.0 (3.8)	0 (0.71)	0.8 (5.1)	1.7 (7.5)	5.2 (13.0)	2.6 (8.7)
Econeem plus® -2.5 ml	60 (7.9)	0 (0.71)	0 (0.71)	0 (0.71)	15.0 (3.8)	0 (0.71)	0.6 (4.4)	1.3 (6.5)	2.5 (8.2)	1.4 (6.4)
Econeem plus ®- 5.0 ml	70 (8.6)	0 (0.71)	0 (0.71)	0 (0.71)	17.5 (4.1)	0 (0.71)	0.0 (0.7)	0.3 (3.0)	1.2 (5.8)	0.5 (3.3)
Neemazal ® -0.75ml	30 (5.0)	0 (0.71)	0 (0.71)	0 (0.71)	7.5 (2.8)	0 (0.71)	0.0 (0.7)	0.8 (5.1)	1.3 (6.1)	0.7 (3.9)
Neemazal® -1.5 ml	30 (3.4)	0 (0.71)	0 (0.71)	0 (0.71)	5.0 (2.1)	0 (0.71)	0.0 (0.7)	0.0 (0.7)	0.5 (3.3)	0.2 (2.1)
Deltamethrin- 40 mg	20 (4.7)	100 (10.0)	860 (29.3)	987 (31.4)	491.8 (22.2)	0 (0.71)	0.0 (0.7)	95.5 (77.9)	100 (90.0)	65.2 (53.8)
Control	60 (7.5)	150 (12.2)	180 (13.3)	240 (15.5)	157.5 (12.6)	0 (0.71)	3.4 (10.3)	27.5 (31.6)	41.5 (40.1)	24.1 (29.4)
CD @ 5%	**2.33**	**1.26**	**1.21**	**1.45**	**1.05**	**NS**	**2.0**	**2.8**	**3.8**	**4.2**

*** Figures in parenthesis are arcsine transformed values**
**** Figures in parenthesis are square root transformed values**

There have been few many studies reported on the ovipositional deterrent action of neem products against the pulse beetle. Khaire et al. (1993) reported that seeds treated with neem oil had a repellent action against egg laying activities of adult beetles. Pandey et al. (1986) also found that plant extracts of neem leaves and twigs gave a high repellent action against *C. chinensis.* Khaire et al. (1993) reported that treating pigeon pea seeds with neem oil showed significant repellent action against egg laying by adult *C. chinensis* beetles for up to 100 days after treatment.

Effect of neem formulations on pulse beetle damage

The percent damage in bengalgram seeds treated with different neem formulations at different periods of storage was found to be significant (Table1). There was no damage recorded in any of the treatments including control up to three months of storage. Except Neemindia (2.2 %) all other neem formulations and chemical check deltamethrin recorded less pulse beetle damage (<1 %) up to six months of storage period compared to untreated control (3.4 %). But nine months after storage, highest damage of 95.5% was recorded with deltamethrin treated seeds which is higher than untreated control (27.5%) where as the neem formulations recorded significantly low insect damage (<1%) except with Neemindia at 2.5 ml (2.2 %). Neemazal and Econeem maintained their superiority with less insect damage (0.5% and 1.2% respectively) even after 12 months of storage compared to 100% damage recorded with deltamethrin and 41.5% damage with untreated control. The cumulative insect damage after twelve months of storage indicated significant differences in different treatments. All neem formulations proved effective against pulse beetle in stored Bengalgram compared to chemical check deltamethrin and untreated control. Surprisingly deltamethrin recorded highest damage (65.2%) which is more than untreated control (24.1%). This may be an indication of resistance development in *C.chinensis* against synthetic pyrethroid deltamethrin due to its indiscriminate usage in stored grains.

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The present research findings are in agreement with the earlier researchers who have reported the efficacy of neem products on pulse beetle damage in stored pulses (Yadav 1985, Sujatha and Punnaiah 1985, Das and Karim, 1986). Studies on the residual effect of neem against *C. chinensis* conducted by Choudhary (1990) showed that the damage by this beetle was reduced on chickpea. Jacob and Sheila (1990) reported that the effectiveness of neem oil against *C. chinensis* on green gram resulted in >60% mortality of the bruchid after 3days. Studies conducted by Pandey and Singh (1995) showed that neem leaf powder could effectively protect black gram seed from damage of *C. chinensis*.They also found that neem bark powder was effective in reducing the damage. Mansour (1997) tested NeemAzal-S against *C. chinensis* in the laboratory and found that 0.5% NeemAzal-S gave 100% mortality of different stages of the pest up to three months.

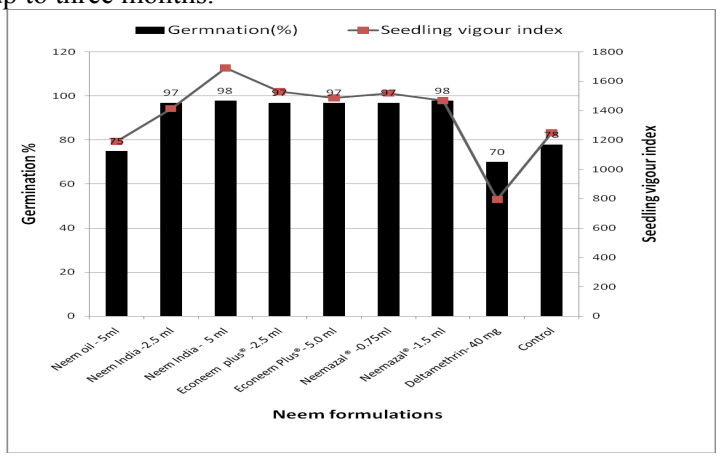

Fig.2. Effect of neem formulations on germinability and seedling vigour index of Bengal gram

Effect of neem formulations on seed germination

Among the different formulations, seeds treated with Neemazal registered the highest seed germination (100, 99, 97 and 95% respectively at three, six, nine and twelve months after storage, respectively). Except crude neem oil (55%), all other neem formulations recorded highest seed germination (>90%) compared to untreated control (50%) and chemical check deltamethrin (30%) after twelve months of storage of bengalgram (Table 2 and Fig.2). The germination of neem oil treated bengalgram seeds were dropped to 77.0 % within six months of storage which is less than the germination of untreated seeds (87.0 %). The pooled mean data of 12 months of storage indicated that Neem formulations *viz.,* Neemazal , Econeemplus and Neemindia at both the test doses recorded high germination (>90%) and proved significantly superior to crude neem oil (75%), chemical check deltamethrin (70%) and untreated check (78%). Similar reports of reduced germination of pulse seeds due to neem oil treatment was reported by Vijayalakshmi and Goswami (1986). In contrary, Patil and Tandale (1999) in greengram and Sreeramaiah and Bommegouda (1992) in cow pea reported that neem oil at 0.5 % maintained higher germination and seed vigor during storage.

Effect of neem formulations on seedling vigor index

The studies on seedling vigor index of bengalgram seed at different storage intervals indicated that the vigor of seedlings decreased with the increase of storage period. The cumulative mean data of 12 months of seed storage indicated significant differences in seedling vigor index of treated seeds at different storage intervals (Table 2 and Fig.2). Neemindia, Neemazal and Econeem plus proved significantly superior to crude neem oil (1190) with highest seedling vigour (1691, 1519 and 1530, respectively). Chemical check deltamethrin treated seeds, due to its poor germination and high insect damage, recorded lowest seedling vigor index (793) which is lower than untreated control (1249). Crude neem oil treatment to bengalgram gram seed resulted in not only poor germination (75%) but also less vigor of the seedling (1190).

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Table 2. Effect of neem formulations on the germination and seedling vigour index of bengalgram

Treatments (ml/kg seed)	Germination %* (months after storage)					Seedling vigour index** (months after storage)				
	3	6	9	12	Mean	3	6	9	12	Mean
Neem oil - 5ml	99.0 (85.4)	77.0 (61.4)	68.0 (55.5)	55.0 (47.8)	75.0 (60.1)	1438 (37.9)	1178 (36.7)	1151 (33.9)	992 (31.5)	1190 (34.5)
Neemindia® -2.5 ml	100 (90.0)	97.0 (80.5)	96.0 (80.7)	94.0 (76.4)	97.0 (80.5)	1439 (37.9)	1472 (38.1)	1375 (37.1)	1365 (36.9)	1413 (37.6)
Neemindia® - 5 ml	99.0 (85.4)	99.0 (85.4)	98.0 (83.4)	96.0 (80.7)	98.0 (82.1)	1950 (44.2)	1619 (42.8)	1601 (40.0)	1594 (39.9)	1691 (41.1)
Econeem plus® -2.5 ml	100 (90.0)	99.0 (85.4)	97.0 (80.5)	92.0 (73.7)	97.0 (80.5)	1636 (40.4)	1566 (40.2)	1473 (38.4)	1445 (38.0)	1530 (39.1)
EconeemPlus®- 5ml	99.0 (85.4)	98.0 (82.1)	97.0 (80.1)	95.0 (79.5)	97.0 (80.5)	1716 (41.5)	1450 (40.3)	1397 (37.4)	1386 (37.)	1487 (38.5)
Neemazal ® -0.75ml	100 (90.0)	98.0 (81.9)	97.0 (80.5)	93.0 (74.9)	97.0 (80.1)	1619 (40.2)	1575 (40.0)	1548 (39.3)	1332 (36.5)	1519 (38.9)
Neemazal® -1.5 ml	100 (90.0)	99.0 (85.4)	97.0 (80.1)	95.0 (79.5)	98.0 (83.4)	1731 (41.6)	1501 (40.6)	1437 (37.9)	1201 (34.6)	1468 (38.3)
Deltamethrin- 40 mg	100 (90.0)	91.0 (72.6)	60.0 (50.8)	30.0 (33.1)	70.0 (56.8)	1732 (41.6)	1438 (40.4)	0.0 (0.71)	0.0 (0.71)	793 (28.1)
Control	100 (90.0)	87.0 (68.9)	76.0 (60.8)	50.0 (45.0)	78.0 (62.1)	1670 (40.9)	1458 (40.0)	1046 (32.3)	823 (28.7)	1249 (35.3)
CD @ 5%	**4.3**	**5.3**	**7.4**	**10.8**	**5.7**	**0.1**	**1.6**	**0.8**	**0.86**	**0.29**

*** Figures in parenthesis are arcsine transformed values**
**** Figures in parenthesis are square root transformed values**

Toxicity of neem based formulations have also been reported by earlier workers on various neem extracts which have repellent, anti feedant and toxic effects against a number of stored grain insect pests (Nazli et al. 2003). Ahmed et al (2000) reported the efficacy of neem extracts compared with cypermethrin and methyl parathion against the stored grain insect pests and observed that neem extracts gave highest mortality of *T. castaneum*, this supports our results of neem formulations especially which gave less insect damage and oviposition of the beetles. Zahid *et al* (2000) reported high (63%) mortality by neem oil comparatively at par with actellic 50EC and malathion 57 EC. The present results receive support from El-Lakwah and El- Kashlan (1999) who reported that Neemazal-W (a powder formulation) gave mortality and reduction in progeny of five major pests of stored grains including *T. castaneum.*
In the present study, the potential of commercial neem formulations like Neemazal, Neemindia and Econeemplus in the storage of pulses and can be used as alternatives to conventional insecticides like deltamethrin for long term safe storage of pulses. These neem products are safe, cheap, residue free and eco- friendly materials that can fit into the IPM package of stored grain pests of pulses.

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