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R. Shanmugapriyan
Lucine Agro Tech India PVT. Ltd., Coimbatore, Tamilnadu, India.

V. Dhanalakshmi
NGM College, Pollachi, Coimbatore District, Tamilnadu, India

Correspondence:
R. Shanmugapriyan
Lucine Agro Tech India PVT. Ltd., Coimbatore, Tamilnadu, India.

Biological effects of neem on the pupation of *Henosepilachna vigintioctopunctata* Fab. on bittergourd

R. Shanmugapriyan and V. Dhanalakshmi

Abstract
Neem derivatives were experimented for their effect on the pupae of *Henosepilachna vigintioctopunctata* (Fabricius) on bittergourd. Neem oil at 2.5 percent concentration demonstrated 53.33 percent mortality of pupae. The pupal mortality ranged from 13.3 to 50.0 percent when the pupae were topically treated with neem seed kernel extract at the concentration range of 0.25 to 2.5 percent. The efficacy of neem seed coat and bark powder extracts were lesser than neem oil, seed kernel and cake extracts. The malformation effect of neem oil at 2.5% was 26.6 percent. The malformation effect of neem oil is followed by neem seed kernel and cake extract. Synthetic insecticides did not prove their effect on adult malformation. Neem oil resulted in 80.0 percent of inhibition on adult emergence at 2.5 percent concentration. The order of the pupicidal effect of neem derivatives was neem oil > seed kernel > cake > seed coat and bark powder extract.

Keywords: Adult malformation, Botanicals, *Henosepilachna vigintioctopunctata* (Fab.), Pupicidal effect, Total inhibition.

1. Introduction
Henosepilachna vigintioctopunctata (Fabricius) is a fairly common coccinellid pest throughout the country and causes considerable damage to a number of plants. The grubs and adults feed on the epidermal tissue of leaves, flowers and fruits [7, 4]. This is a serious pest of various economically important vegetable crops and has also developed resistance to almost all commercially available synthetic pesticides [1]. Plant derived pesticides are highly safe to the environment. They are easily biodegradable, lack of persistence in agricultural produce and bioaccumulation in the ecosystem, which have been key problems in synthetic insecticides. The neem derivatives have shown their insecticidal properties on a broad range of insect pests [8]. Azadirachtin showed insect growth regulating activity against many insects [20, 13]. Neem oil at 3.0 percent concentration reduced the pupation of *Arthalia proxima* [17]. Application of neem oil and neem cake extract on the pupae of gall midge, *Orseolia oryzae* was highly effective [11]. Abnormal adult emergence, death in pre pupal stage, deformation and inactivation of pupation was recorded while treating the prepupae and pupae of *Helicoverpa armigera* with neem seed powder [6]. Neem seed kernel extract and neem oil significantly inhibited the growth and development of *Spodoptera litura* and extended the pupal period [15]. Neem oil at 2, 3 and 5 g/l affected the pupal survival of *Bactrocera oleae* [24]. The present study was conducted to evaluate the pupicidal effect of aqueous extracts of neem oil, cake, seed kernel, seed coat and bark powder against the pupae of *Henosepilachna vigintioctopunctata* (Fab.) on bittergourd.

2. Materials and Methods
The pupicidal effect of neem oil, extracts of neem cake, seed kernel, seed coat and bark powder at 0.25, 0.5, 1.5 and 2.5% concentrations along with thiamethoxam 25 WG @ 0.025%, quinalphos 25 EC @ 0.05%, chlorpyriphos 20 EC @ 0.05% and dimethoate @ 0.05% were evaluated against the pupae of *H. vigintioctopunctata* (F.). Egg masses were collected from the bittergourd field and reared in the laboratory of Zoology department of NGM College, Pollachi at 26±2 ºC between April to October 2014. Each day insect culture box was cleaned and provided with fresh bittergourd leaves. Ten number of healthy fourth instar larvae were transferred into a petri dish and allowed to pupate. Test solutions were prepared separately with tap water and sprayed on the sedentary pupae with a glass atomizer. The solvent was left to evaporate under an electric fan for twenty minutes.

The air dried, treated pupae with petri dishes were kept in to a well ventilated transparent plastic box. There are three replications, each replication has ten pupae. The observations were made on pupal mortality, malformed adults, normal adults and total inhibition of adult emergence. The data were statistically analyzed with the help of SPSS computerized software (version 20) for Duncan's multiplication range test (DMRT) at 5% level.

3. Results and Discussion

When the pupae were treated with neem oil in the concentration range of 0.25 to 2.5 percent, the pupal mortality ranged between 20.0 and 53.3 percent and 26.6 percent and inhibition on adult emergence varied between 33.3 and 80.0 percent (Table-1). Neem oil caused 26.6 percent malformation during adult emergence. Incorporation of leaf extract of *Murraya koenigii* with neem oil reduced the adult emergence of *Callosobruchus maculates* [18]. The efficacy of neem oil on the pupal survival of *Spodoptera litura* was recorded [5]. Reduced pupation was observed in *Arthalia proxima* when treated with neem oil at 0.4 to 3.0 percent [15]. The presence of azadirachtin in neem extracts was the main causative agent for the pupal growth retardation and malformation [8]. Application of azadirachtin on bentgrass resulted moulting disorders and stunted growth in *Agrotis ipsilon* [3].

Neem cake extract at the concentration range of 0.25 to 2.5% offered 23.3 to 36.6 percent pupal mortality. 2.5 percent neem cake extract resulted in 20.0 percent of malformed adults. Total inhibition of adult emergence due to the treatment of neem cake extract was 56.6 percent. High pupal mortality was registered when the pupae of *Orseolia oryzae* were treated with 2.0 percent neem cake extract [11]. Neem cake extract at 5.0 percent disturbed normal adult emergence upto 26.6 percent in *Dysdercus cingulatus* [25] and inhibited the adult emergence upto 40.0 percent when sprayed with aqueous neem cake extract.

When the pupae were treated with neem seed kernel extract 0.25, 0.5, 1.5 and 2.5%, the pupal mortality ranged between 13.3 and 50.0%, adult malformation varied from 10.0 to 23.3 percentage, normal adult formation from 76.6 to 26.6% and total inhibition ranges from 23.3 to 73.3 percent. Earlier studies revealed the pupicidal effect of neem seed extract at 0.02 percent when experimented against *Callosobruchus chinensis* [22]. Application of neem oil and seed kernel extracts significantly inhibited the growth and development of *Helicoverpa armigera* and *Spodoptera litura* [6, 15]. Higher concentration of neem seed kernel extract (6.0%) reduced the percentage of the adult emergence of cowpea aphid, *Aphis craccivora* (Koch) [19]. Neem seed kernel extract at 2 to 10 ppm concentration showed 18 to 56 % and 6 to 34 % pupal death in *Anopheles stephensi* and *Chironomus circumdatus* [10]. There was an inverse relationship between the dose and the efficacy of neem seed kernel extract on the development of pupal malformation and the emergence of *Anticarsia gemmatalis*. Abnormal adult emergence was inversely proportional to the increasing dose of extract of neem seed kernel. Adults did not emerge from treated pupae [2]. Blending of neem seed kernel extract with leaf extract *Murraya koenigii* was the most effective on the infestation of cowpea weevil, *Callosobruchus maculatus* (F) and results least number of adult emergence over other treatments [18]. Neem seed powder act as insect growth regulator against the red flour beetle, *Tribolium castaneum*. The rising trend of growth inhibition was recorded with increasing the dose of neem seed powder from 0.5 to 2.0% [23]. The pupal mortality of red flour beetle, *T. castaneum* was 49.67, 68.12 and 69.43 percent due to the treatment of water extracts of neem seeds stored in the sun, refrigerator and room [21].

The pupal mortality ranged from 6.6 to 43.3 and 6.6 to 20.0%, when the pupae were treated with neem seed coat and bark powder extract. Inhibition of the adult emergence of treated pupae was 13.3 to 50.0 and 6.6 to 30.0 while treating with neem seed coat and bark powder extracts. The pupal mortality effect of all neem derivatives increased with an increase in their concentration. This is in accordance with the earlier findings on the pupicidal effect of neem oil against *Arthalia lugens* [17]. The pupal mortality of *Aedes aegypti* also decreased with decreasing concentrations of extracts of neem seed kernel [26]. A significant declining trend of adult emergence with increased dose of neem leaf powder and 100 percentage pupal mortality was attained when *Corcyra cephalonica* were treated with 3 percentage neem leaf powder [16]. Effect of neem oil and neem seed kernel extract on pupation was well exhibited in *Anopheles stephensi* [14]. It was reported that the percentage of pupation and adult emergence was dose dependent and no pupation recorded with 5 percentage neem oil treatment. These findings corroborated with the earlier research done on *Orseolia oryzae and Bactrocera oleae* [11, 24].

At lower concentrations of neem leaf extracts (1, 5 and 10%), pupation of *Culex pipiens fatigans* was significantly delayed by two or three days whereas in higher concentration (40, 50 and 60%) resulted a complete arrest of pupation [12]. Azadirachtin at 1, 5, 10 or 25 ppm on bean plants greatly affected the pupal development and adult moulting of larvae. A soil drench of azadirachtin resulted adverse effect on pupation of *Liriomyza huidobrensis* and molting than leaf dipping treatment [27]. In the present study, thiamethoxam, quinalphos, chlorpyriphos and dimethoate registered 80.0, 73.3, 33.3 and 36.6 % of pupal mortality against the pupae of epilachna beetle. When the pupae were treated with 0.025% thiamethoxam 25 WG and 0.05% of quinalphos, chlorpyriphos and dimethoate, the inhibition of adult emergence was 83.3, 73.3, 33.3 and 36.6%.

In the present study, neem oil was superior to the rest of the extracts. The order of the pupicidal effect of neem derivatives was neem oil > seed kernel cake > seed coat and bark powder extract. Adult emergence effect of neem oil was higher than neem cake extract when tested against *Dysdercus cingulatus* [25]. Thiamethoxam was highly effective on the pupation and the efficacy was higher than the botanicals and other synthetic insecticides. Neem derivatives exhibited malformation effect on the adults. Synthetic insecticides did not produce malformation effect on the pupae. Thiamethoxam superiorly affected the inhibition of adult emergence. The efficacy of thiamethoxam on the pupae of epilachna beetle was followed by neem oil, seed kernel extract, quinalphos, neem cake, seed coat extract, dimethoate and chlorpyriphos.

Table 1: Pupicidal effect of neem derivatives on *H. vigintioctopunctata* (Fab.)

Treatment (%)	Pupal mortality (%)	Malformed Adults (%)	Normal Adults (%)	Total inhibition (%)
Neem oil @ 0.25	20.0000 (26.5674) e-h	13.3333 (21.1449) a-d	66.6666 (54.7821) c-g	33.3333 (35.2177) e-h
Neem oil @ 0.5	30.0000 (33.0024)c-g	16.6666 (23.8550)abc	53.3333 (46.9227)d-h	46.6666 (42.9930)c-g
Neem oil @ 1.5	43.3333 (41.1542)cde	23.3333 (28.7803)a	36.6666 (37.1405)g-j	70.0000 (56.9974)a-d
Neem oil @ 2.5	53.3333 (46.9227)bc	26.6666 (30.9955)a	20.0000 (26.0702)ij	80.0000 (63.9296)ab
Neem Cake Extract @ 0.25	23.3333 (28.7803)d-h	0.0000 (0.1910)f	76.6666 (61.2195)b-e	23.3333 (28.7803)f-i
Neem Cake Extract @ 0.5	30.0000 (33.21)c-g	6.6666 (12.2899)b-f	63.3333 (52.7753)c-g	36.6666 (37.2245)e-h
Neem Cake Extract @ 1.5	33.3333 (35.2177)c-g	16.6666 (23.3602)abc	50.0000 (44.9159)e-i	50.0000 (45.0839)b-f
Neem Cake Extract @ 2.5	36.6666 (37.2245)c-f	20.0000 (26.5674)a	43.3333 (41.1542)f-i	56.6666 (48.8456)b-e
Neem Seed Kernel Extract @ 0.25	13.3333 (17.7100)gh	10.0000 (11.0703)c-f	76.6666 (66.1449)bcd	23.3333 (23.8550)ghi
Neem Seed Kernel Extract @ 0.5	20.0000 (26.0702)e-h	13.3333 (21.1449)a-d	66.6666 (55.0746)c-g	33.3333 (34.9252)e-h
Neem Seed Kernel Extract @ 1.5	40.0000 (39.1474)cde	20.0000 (26.0702)ab	40.0000 (38.8549)ghi	60.0000 (51.1449)b-e
Neem Seed Kernel Extract @ 2.5	50.0000 (44.9159)bcd	23.3333 (28.7803)a	26.6666 (29.9999)hij	73.3333 (59.9999)abc
Neem Seed Coat Extract @ 0.25	6.6666 (12.2899)hi	0.0000 (0.1910)f	93.3333 (77.7099)ab	6.6666 (12.2899)ij
Neem Seed Coat Extract @ 0.5	13.3333 (21.1449)fgh	0.0000 (0.1910)f	86.6666 (68.8549)bc	13.3333 (21.1449)hi
Neem Seed Coat Extract @ 1.5	20.0000 (26.0702)e-h	3.3333 (6.1450)ef	76.6666 (61.9227)b-e	23.3333 (28.0771)f-i
Neem Seed Coat Extract @ 2.5	43.3333 (41.1542)cde	6.6666 (8.8550)def	50.0000 (44.9999)e-i	50.0000 (44.9999)b-f
Neem Bark Powder Extract @ 0.25	6.6666 (12.2899)hi	0.0000 (0.1910)f	93.3333 (77.7099)ab	6.6666 (12.2899)ij
Neem Bark Powder Extract @ 0.5	13.3333 (21.1449)fgh	3.3333 (6.1450)ef	83.3333 (66.1448)bcd	16.6666 (23.8550)ghi
Neem Bark Powder Extract @ 1.5	16.6666 (19.2221)gh	6.6666 (12.2899)b-f	76.6666 (62.7099)b-e	23.3333 (27.2899)f-i
Neem Bark Powder Extract @ 2.5	20.0000 (21.1449)fgh	10.0000 (18.4381)a-e	70.0000 (58.0770)c-f	30.0000 (38.0677)d-h
Thiamethoxam @ 0.025	80.000 (67.8593)a	3.3333 (6.1450)ef	16.6666 (19.9253)j	83.3333 (70.0746)a
Quinalphos @ 0.05	73.3333 (59.2127)ab	0.0000 (0.1910)f	26.6666 (30.7871)hij	73.3333 (59.2127)abc
Chlorpyriphos @0.05	33.3333 (35.2177)c-g	0.0000 (0.1910)f	66.6666 (54.7821)c-g	33.3333 (35.2177)e-h
Dimethoate @ 0.05	36.6666 (37.2245)c-f	0.0000 (0.1910)f	63.3333 (52.7753)c-g	36.6666 (37.2245)e-h
Control	0.0000 (0.1910)i	0.0000 (0.1910)f	100.0000 (89.8089)a	0.0000 (0.1910)j

Values are mean of three replications

Means followed by the same letter are not significantly different at the 5% level by DMRT

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