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Fly control efficacy of neem products in comparison with methoprene against the house fly (*Musca domestica*) in experimental broilers farm

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Abstract

The fly control efficiency of neem products (Neem seed powder and Neem seed oil) against house fly was evaluated in experimental broiler shed in comparison with methoprene. One hundred and ninety two, day old broiler chicks were randomly divided into eight groups of twelve each with two replicates and the study was conducted for a period of five weeks. Methoprene and neem seed powder were given through the feed @ of 10 gm and 1 Kg per tonne of feed, respectively and neem oil (5% v/v) was sprayed on broiler litter material twice a week until the completion of the study. The body weight and body weight gain were recorded at weekly intervals in all treatment groups. Fly larval count was measured on 7th, 14th, 21st, 28th and 35th days and pupicidal activity was observed on 21st day by *in vitro* method. There was no significant change in body weight and body weight gain on inclusion of neem seed powder and methoprene at first, second, third and fourth weeks whereas neem seed powder inclusion in the feed exhibited significant decrease in the body weight and body weight gain at fifth week. All the feed incorporated treatments possessed good larvicidal activity on 7th, 14th, 21st, 28th and 35th days. Mere spraying of neem oil possessed larvicidal effect but not in comparable level with methoprene or neem seed powder supplemented diets of broilers. Pupicidal activity was significant in neem oil sprayed group compared to that of control. It may be reasonably concluded that dietary inclusion of neem seed and neem oil spray in litter material showed good fly control effect without much affecting production parameters. The efficacy of neem products was comparable with that of methoprene and combination of neem products with methoprene was synergistic.

Keywords: Fly control, methoprene, neem seed powder, neem oil, broiler birds

Introduction

The common housefly, *Musca domestica* L, has made some considerable effects in the poultry industry, as it can spread approximately 100 pathogenic organisms, cause nuisance to workers and deposit dirt and microorganisms, this results in losses in egg and meat production. A housefly can fly several kilometers, visiting homes, settling on food and excreta and invade aviaries and other local animal creations and hence the species is considered a serious problem for public health, livestock and poultry ^[1]. The control of this insect largely relies on synthetic insecticides which have led to many serious issues like resistance development, ecological imbalances, bioaccumulation and harm to non-target organisms, environmental contamination and incorporation in to food chains ^[2]. Therefore, more attention has been recently paid to the use of natural or organic insecticides such as insect growth regulators (IGRs) and natural plant based products for controlling housefly in different parts of the world ^[3].

Methoprene is one of the existing IGR which affects exclusively the flies without any significant toxic damage upon their natural enemies. Methoprene products have been developed which act especially upon their first instar, hindering their aptitude to moult to the next stage and disabling pupae to reach the adult stage. Moderate to high resistance to methoprene develops if the selection pressure is strong enough, as in the case of feed-through treatment. Hence, producers must rely on alternative strategies for fly control. Among the economically viable bioinsecticides, neem (*Azadirachta indica*) stands out as it is less polluting and has low residual power and risk of toxicity to mammals and birds ^[4]. This product generally has been used in integrated pest management to control flies. Its active ingredient is a triterpenoid compound, azadirachtin, showing various lethal and sublethal effects on insects, including oviposition and feeding deterrence, growth regulation and fecundity ^[5].

Neem is active against house fly and other livestock pests ^[6]. Neem formulations can be standardized and used as best insecticides for fly control in poultry farming operations, because of the absence of resistance development, lack of residues, environmental safety, ready availability and cost effectiveness ^[7]. Therefore, the study was designed with an aim of assessing the effect of neem products i.e, neem seed powder and neem seed oil in the control of house flies in comparison with methorprene in broilers.

Materials and Methods

Drugs and chemicals

Methoprene was obtained *as gratis* from M/s. Nutricon Company, Chennai. Neem seeds were collected from Veterinary College and Research Institute, Namakkal campus and then shade dried. Neem oil was obtained from local commercial market in Namakkal. Soft soap (10% v/v) solution was used as emulsifier. Shed of Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Namakkal, in broiler chickens. One hundred and ninety two, day-old commercial broiler chicks of straight run obtained from M/s TPK traders at Coimbatore were utilized in this biological trial.

On arrival of chicks, they were weighed, wing banded and randomly assigned to eight treatment groups with two replicate of twelve chicks each. The birds were reared in table-top cages under standard management practices and the experiment was conducted from one day to thirty five days. The birds were fed with standard broiler ration without antibiotics and toxins from feed manufacturing technology unit of Veterinary College and Research Institute, Namakkal. The birds had access to *ad libitum* feed and water throughout the study period. The experiment was approved by the Institutional Animal Ethical Committee of Veterinary College and Research Institute, Namakkal (Approval no: IAEC/11/VCRI-NKL/2019). The details of experimental design was presented in Table 1.

Birds and experimental design

The present study was conducted at the Experimental Poultry

Table 1: Experimental design of the study

Group	Treatment	No. of chicks (2x24)	Route of administration	
T1	Control	24	Only basal diet	
T2	Methoprene (10 gm per tonne of feed)	24	Through feed, throughout the study period	
T3	Neem seed powder (1 kg per tonne of feed)	24	Through feed, throughout the study period	
T4	Neem oil spray (5% v/v)	24	As spray on litter material (twice a week)	
T5	Methoprene (10 gm per tonne of feed) + Neem seed (1 kg per tonne of feed)	24	Through feed, throughout the study period	
T6	Methoprene (10 gm per tonne of feed) + Neem oil spray (5% v/v)	24	Methoprene – through feed; Neem oil spray on litter material	
T7	Neem seed powder (1 kg per tonne of feed) + Neem oil Spray (5% v/v)	24	Neem seed powder – through feed; Neen oil–spray on litter material	
Т8	Methoprene (10 gm per tonne of feed) + Neem seed powder (1 kg per tonne of feed) + Neem oil Spray (5% v/v)	24	Methoprene and Neem seed powder– through feed; Neem oil – spray on litter material	
	Total	192		

Production parameters

The weekly body weight, body weight gain and final body weight was evaluated for five weeks.

Fly larval count

The viability of the fly larvae was evaluated in the chicken faecal material below each group as per Silva *et al.* ^[8]. The faecal material below the cages in each group was randomly subdivided into six equal areas. Samples of 100 gm of faeces from each subdivided area were taken on 7th, 14th, 21st, 28th and 35th days during treatment and the fly larvae was counted.

Pupicidal activity

The pupicidal activity was evaluated *in vitro* by spraying neem oil on the larva collected from control group as per the method of Siriwattanarungsee *et al.* ^[9]. Fresh third instar larvae (thirty numbers) were collected from control group and were introduced into separate transparent plastic boxes and provided with broiler feed. The top area of the plastic box was covered with an aerated lid for ventilation. Neem oil emulsion (5% v/v in 10% soap solution) was sprayed on 3rd day and the pupa was counted on 5th day in the treatment group. Control group was treated with soap solution (vehicle) similar to treatment group. This experiment was replicated six times and treatment group was compared with control.

Statistical analysis

Data were analyzed by ANOVA and Student's t-test and means were compared with Tukey's post-hoc test using SPSS16 software. Data were expressed as mean \pm SE. A value of p< 0.05 was considered statistically significant.

Results

Body weight

The result on body weight (g) of broilers belonging to different groups is presented in Table 2. The mean body weight (g) of day old birds of groups I to VIII were 50.562 ±0.57, 50.917 ±0.58, 49.938±0.85, 50.812±0.70, 50.896±0.57, 49.854±0.67, 51.062±0.58 and 49.917±0.67 respectively. No significant differences were observed in body weight among the groups. The mean first week body weight (g) of different treatment groups I to VIII were 168.04 ± 2.68 , 170.04 ± 1.87 , 167.79 ± 2.33 , 181.04 ± 2.12 , 168.41 ± 2.66 , 171.16 ± 1.81 , 172.04 ± 2.73 and 177.83 ± 2.08 respectively. No significant differences were observed in body weight among the different treatment groups. The mean second week body weight (g) of different treatment groups I to VIII were 405.04 ± 5.42 , $404.16 \pm 3.65, 392.29 \pm 3.93, 411.08 \pm 4.06, 405.20 \pm 4.17,$ 403.12 ± 3.93 , 388.95 ± 3.77 and 398.042 ± 3.39 respectively. No significant differences were observed in body weight among the different treatment groups. The mean third week

body weight (g) of different treatment groups I to VIII were 703.58 \pm 3.58, 704.08 \pm 4.03, 688.33 \pm 2.95, 710.95 \pm 2.67, 701.75 \pm 3.64, 704.91 \pm 3.97, 690.00 \pm 3.48 and 699.37 \pm 3.98 respectively. No significant differences were observed in body weight among the different treatment groups. The mean fourth week body weight (g) of different treatment groups I to VIII were 1151 \pm 25.65, 1173 \pm 21.66, 1117 \pm 12.45, 1189 \pm 16.55, 1182 \pm 15.94, 1170 \pm 21.10, 1139 \pm 12.71, 1152 \pm 18.40, respectively. No significant differences were observed in

body weight among the different treatment groups. The mean fifth week body weight (g) of different treatment groups I to VIII were 1614 ± 29.59 , 1595 ± 30.84 , 1489 ± 13.21 , 1595 ± 18.75 , 1575 ± 21.93 , 1589 ± 28.78 , 1477 ± 15.89 and 1524 ± 19.52 respectively. Compared to control (T1), there was significant reduction in body weight was noticed in the T3 and T7 treatment groups, wherein neem seed powder was included in the feed.

 Table 2: Effect of dietary inclusion of methoprene, neem seed powder and neem oil spray on litter material on body weight (g) (Mean ± S. E.) of broilers.

Treatment	Day old	I Week	II Week	III Week	IV Week	V Week
T1	50.562 ± 0.57	168.04 ± 2.68^{ab}	405.04 ± 5.42^{ab}	703.58± 3.58 ^{abc}	1151 ± 25.65^{ab}	1614±29.59°
T2	50.917 ± 0.58	170.04 ± 1.87^{ab}	404.16 ± 3.65^{ab}	704.08±4.03 ^{bc}	1173 ± 21.66^{ab}	$1595 \pm 30.84^{\circ}$
T3	49.938 ± 0.85	167.79 ± 2.33^{a}	392.29 ± 3.93^{a}	688.33 ± 2.95^{a}	1117±12.45 ^b	1489±13.21 ^{ab}
T4	50.812 ± 0.70	$181.04 \pm 2.12^{\circ}$	411.08 ± 4.06^{b}	710.95± 2.67°	1189 ± 16.55^{a}	1595±18.75°
T5	50.896 ± 0.57	168.41 ± 2.66^{ab}	405.20 ± 4.17^{ab}	701.75± 3.64 ^{abc}	1182 ± 15.94^{a}	1575±21.93 ^{abc}
T6	49.854 ± 0.67	171.16± 1.81 ^{abc}	403.12± 3.93 ^{ab}	704.91± 3.97 ^{bc}	1170±21.10 ^{ab}	1589±28.78 ^{bc}
T7	51.062 ± 0.58	172.04± 2.73 ^{abc}	388.95 ± 3.77^{a}	690.00 ± 3.48^{ab}	1139±12.71 ^{ab}	1477 ± 15.89^{a}
T8	49.917 ± 0.67	177.83 ± 2.08^{bc}	398.042 ± 3.39^{ab}	699.37 ± 3.98^{abc}	1152 ± 18.40^{ab}	$1524{\pm}19.52^{abc}$

Values (Mean \pm S.E.M., n = 24) in the same column bearing no superscript common vary significantly (P< 0.05) in Tukey's multiple comparison post hoc test.

Body weight gain

The result on the effect of different treatment on body weight gain (g) of broilers is presented in Table 3. The first week body weight (g) gain of broiler birds of groups I to VIII were $117.47 \pm 2.49, 119.12 \pm 1.52, 117.85 \pm 1.95, 130.22 \pm 1.88,$ 117.52 \pm 2.40, 121.31 \pm 1.92, 120.97 \pm 2.48 and 127.91 \pm 2.00 respectively. No significant differences were observed in body weight gain among the different treatment groups. The second week body weight (g) gain of broilers birds of groups I to VIII were 354.47 ± 5.34 , 353.25 ± 3.37 , 342.35 ± 3.57 , 360.27± 3.95, 354.31± 4.11, 353.27± 4.01, 337.89± 3.95 and $348.12\pm$ 3.24 respectively. No significant differences were observed in body weight gain among the different treatment groups. The third week body weight (g) gain of broiler birds of groups I to VIII were 653.02 ± 3.54 , 653.16 ± 3.76 , 638.39 \pm 2.90, 660.14 \pm 2.95, 650.85 \pm 3.63, 655.06 \pm 4.04, 638.93 \pm 3.50 and 649.45 \pm 3.87 respectively. Compared to control (T1), there was significant reduction in body weight was noticed in the T3 and T7 treatment groups, wherein neem seed powder was included in the feed. The fourth week body weight (g) gain of broilers birds of groups I to VIII were $1100\pm 25.55, 1122\pm 21.58, 1067\pm 12.10, 1138\pm 16.56, 1131\pm$ 15.93, $1120\pm$ 21.18, $1088\pm$ 12.65 and $1102\pm$ 18.30 respectively. No significant differences were observed in body weight gain among the different treatment groups. The fifth week body weight (g) gain of broilers birds of groups I to VIII were 1563±29.42, 1544± 30.68, 1439± 12.94, 1544± 18.75, 1524 \pm 21.92, 1539 \pm 28.84, 1426 \pm 15.92 and 1474 \pm 19.53 respectively. Compared to the control group (T1) in which only basal diet was fed and the group fed with basal diet and methoprene (T2), the groups fed with neem seed powder (T3 and T7) along with basal diet had reduction in body weight, which was significant (p < 0.05). Among the groups (T3 and T7) in which neem seed powder was included there was no significant difference.

Table 3: Effect of dietary inclusion of methoprene, neem seed powder and neem oil spray on litter material on body weight gain (g) (Mean \pm S.E.) of broilers

Treatment	I Week	II Week	III Week	IV Week	V Week
T1	117.47 ± 2.49^{a}	354.47 ± 5.34^{ab}	653.02 ± 3.54^{b}	1100 ± 25.55^{ab}	1563±29.42°
T2	119.12 ± 1.52^{ab}	353.25 ± 3.37^{ab}	653.16 ± 3.76^{b}	1122 ± 21.58^{ab}	1544± 30.68°
T3	117.85 ± 1.95^{a}	342.35 ± 3.57^{a}	638.39 ± 2.90^{a}	1067 ± 12.10^{a}	1439±12.94 ^{ab}
T4	$130.22 \pm 1.88^{\circ}$	360.27 ± 3.95^{b}	660.14 ± 2.95^{b}	1138 ± 16.56^{b}	1544± 18.75°
T5	117.52 ± 2.40^{a}	354.31±4.11 ^{ab}	650.85 ± 3.63^{b}	1131±15.93 ^b	1524±21.92 ^{abc}
T6	121.31 ± 1.92 ^{abc}	353.27± 4.01 ^{ab}	655.06 ± 4.04^{b}	1120 ± 21.18^{ab}	1539 ± 28.84^{bc}
Τ7	120.97 ± 2.48^{ab}	337.89 ± 3.95^{a}	638.93 ± 3.50^{a}	1088 ± 12.65^{ab}	1426±15.92ª
T8	127.91 ± 2.00^{bc}	348.12 ± 3.24^{ab}	649.45 ± 3.87^{b}	1102±18.30 ^{ab}	1474 ± 19.53^{abc}

Values (Mean \pm S.E.M., n = 24) in the same column bearing no superscript common vary significantly (P < 0.05) in Tukey's multiple comparison post hoc test.

Fly larval count

The house fly larvae of all groups were counted on 7th, 14th, 21st, 28th and 35th days and presented in Table 4. The mean values of larval count for the groups T1 to T8 on 7th day were 3.17 ± 0.78 , 2.08 ± 0.45 , 2.0 ± 0.60 , 1.67 ± 0.56 , 2.08 ± 0.45 , 1.17 ± 0.32 , 1.92 ± 0.54 and 1.0 ± 0.35 respectively. Compared to control (T1) larvicidal activity was significantly higher in T8 and T6 wherein methoprene and neem seed was

incorporated in the feed and neem oil was sprayed on litter and methoprene included in feed and neem oil was sprayed in litter material, respectively. The mean values of larval count for the groups T1 to T8 on 14th day were 22.42 \pm 2.75, 12.58 \pm 1.5, 12.42 \pm 1.88, 18.67 \pm 2.71, 9.75 \pm 1.74, 10.83 \pm 1.11, 11.38 \pm 1.83 and 6.55 \pm 1.39 respectively. All the treatment groups showed significantly better larvicidal effect as compared to control (T1), except T4 group, wherein neem oil was sprayed in the litter material. The mean values of larval count for the groups T1 to T8 on 21^{th} day were 36.58 ± 4.04 , $24.25 \pm 3.59, 26.50 \pm 3.94, 29.83 \pm 3.88, 14.67 \pm 1.40, 19.33 \pm$ 1.88, 21.85 \pm 3.35 and 9.91 \pm 1.51 respectively. Compared to control (T1), there was no significant difference in larvicidal activity was noticed in the T2, T3, T4 treatment groups. However, larvicidal activity was significantly higher in T8, T5, T6 and T7 wherein methoprene and neem products were given simultaneously with specified combination. The mean values of larval count for the groups T1 to T8 on 28th day were 40.75 ± 3.52 , 27.17 ± 3.81 , 31.83 ± 3.81 , 35.92 ± 2.67 , $16.83 \pm 2.11, \ 20.0\pm 2.86, \ 24.33 \pm 4.26 \ and \ 13.08 \pm 1.67$ respectively. Methprene and neem seed powder inclusion in the feed and neem oil sprayed in the litter material alone treatment groups (T2, T3, T4) did not shown any significant larvicidal effect as compared to control (T1). But different combination treatment groups (T5, T6, T7 and T8) showed

significant larvicidal effect. The mean values of larval count for the groups T1 to T8 on 35^{th} day were 43.50 ± 5.03 , $27.83 \pm 3.85, 31.50 \pm 2.30, 36.75 \pm 4.07, 17.17 \pm 3.26, 20.50 \pm$ 3.31, 24.08 \pm 3.48 and 12.33 \pm 2.77 respectively. Larvicidal efficacy of neem oil spray on poultry litter alone (T4) did not have comparable fly control efficacy as that of either methoprene (T2) or neem seed powder (T3) supplemented diets of broilers. Among the methoprene and neem seed powder supplemented diets, methoprene supplemented diet had better fly control efficacy though it is not significant with the other. Combined effect of neem seed powder and methoprene inclusion in feed (T5) showed better fly control effect than neem seed and methoprene alone treatments. Combined effects of feed supplements along with neem oil spray (T6 & T7) had better fly control effects over the feed supplements alone. There was synergism observed when neem products were combined with methoprene (T8).

 Table 4: Effect of dietary inclusion of methoprene, neem seed powder and neem oil spray on poultry litter on fly larval count (Mean ± S. E.) in broiler manure.

Treatment	7 th day	14 th day	21 th day	28 th day	35 th day
T1	3.17 ± 0.78^{b}	22.42 ± 2.75^{b}	$36.58{\pm}4.04^{d}$	40.75 ± 3.52^{e}	43.50 ± 5.03^{e}
T2	2.08 ± 0.45^{ab}	12.58 ± 1.5^{a}	24.25 ± 3.59^{bcd}	27.17± 3.81 ^{bcde}	27.83 ± 3.85^{bcd}
T3	2.00 ± 0.60^{ab}	12.42 ± 1.88^{a}	26.50 ± 3.94^{bcd}	31.83± 3.81 ^{cde}	31.50 ± 2.30^{cd}
T4	1.67 ± 0.56^{ab}	18.67 ± 2.71^{b}	29.83 ± 3.88^{cd}	35.92±2.67 ^{de}	$36.75{\pm}4.07^{de}$
T5	2.08 ± 0.45^{ab}	9.75 ± 1.74^{a}	14.67 ± 1.40^{ab}	16.83±2.11 ^{ab}	17.17 ± 3.26^{ab}
T6	1.17 ± 0.32^{a}	10.83 ± 1.11^{a}	19.33±1.88abc	20.00±2.86 ^{abc}	20.50± 3.31 ^{ab}
T7	1.92 ± 0.54^{ab}	11.38 ± 1.83^{a}	21.85 ± 3.35^{abc}	$24.33{\pm}4.26^{abcd}$	24.08 ± 3.48^{bc}
T8	1.0 ± 0.35^{a}	6.55 ± 1.39^{a}	9.91±1.51 ^a	13.08 ± 1.67^{a}	12.33 ± 2.77^{a}

Values (Mean \pm S.E.M., n = 6) in the same column bearing no superscript common vary significantly (P < 0.05) in Tukey's multiple comparison post hoc test.

Pupicidal activity

The house fly pupa were counted by *in vitro* method on 5th day of treatment and presented in Table 5. The mean values of abnormal / dead pupa for the control group and treated group (neem oil spray) were 2.0 ± 0.45 and 12.5 ± 0.67 , respectively. Significant difference between the treatment group and control group was observed.

Table 5: Pupal count (Mean \pm SE) as influenced by spraying ofneem oil on litter material by *in vitro* method

Groups	5 th Day	% of pupicidal activity
Control	2.0 ± 0.45^{a}	6.6
Treatment	12.5 ± 0.67^{b}	41.6

Values (Mean \pm S.E.M., n = 6) in the same column bearing no superscript common vary significantly (*P*< 0.05) in Tukey's multiple comparison post hoc test.

Discussion

The present study was undertaken to evaluate the fly control effects of neem products i.e. neem seed powder and neem oil either alone or in combination and comparison with methoprene, in experimental broiler farm. There is extreme paucity of research on effect of neem products combined with methoprene on body weight and body weight gain of broilers. In the present study, methoprene at the dose rate of 10 gram per tonne of feed was fed to broiler bird and found no significant changes in body weight and body weight gain. Dietary inclusion of neem seed powder at the dose rate of 1 kg per tonne of feed to broilers birds showed significant reduction in body weight and body weight gain at 5th week. These results are inconsistence with Landy *et al.* ^[10], where in they observed a significant decline in the average of body weight when adding a powder neem leaves at a rate of 7 and

12 grams/kg to broiler diet at age of 42 day, the reason could be attributed to bitter taste of neem.

In the present study, fly larval count for larvicidal activity was compared with all treatment groups on 7th, 14th, 21st, 28th and 35th day. All the feed incorporated treatments showed significant larvicidal activity than that of control. Larvicidal activity of neem oil spray (T4) did not differ significantly from control. Combined effect of neem seed powder and methoprene inclusion in feed (T5) showed better fly control effect than neem seed and methoprene alone treatments. Combined effects of feed supplements along with neem oil spray (T6 & T7) had better fly control effects over the feed supplements alone. There was synergism observed when neem products were combined with methoprene (T8). From this observation, it was found that larvicidal activity was more effective when methoprene alone was added to the feed. This is in accordance with the findings of Morgan et al. [11] who have reported based on laboratory bioassays, a technical formulation of methoprene applied as a feed additive reduced the fly emergence to 4% or less with a 50.0 ppm or 1% or less with a 100 ppm rate.

Administration of neem seed powder through feed caused highest larvicidal activity and it might be due active compounds of neem which had insect growth regulation and repellency against insects. This is in accordance with Dua *et al.* ^[12] who stated that neem seeds contain approximately 99 biologically active compounds of which azadirachtin, nimbin, nimbidin, and nimbolides as major molecules. Many of these products besides acting as insect growth regulators and fly repellent do have antifeedant, ovicidal and fecundity suppression effect. These kill insects by many different methods and hence possesses good insecticidal effect. Administration of neem products through feed and spraying neem oil in litter material caused highest larvicidal activity. This is in accordance with the study of Akinwale *et al.* ^[13] who found that the powders of ripe and unripe neem seed powder have insecticidal effects against all the reproductive stages and as well as the adult male and female housefly, *Musca domestica*. When compared with the control diet, the neem powders significantly reduced the female fecundity and delayed first egg laying. The pupicidal activity was observed *in vitro* with third instar larva against neem oil spray on 5th day. Neem oil treated group showed higher pupicidal activity compared to control group. This concurs with the study of Rovida *et al.* ^[14] who stated that *Musca domestica* larva treated with 0.5%, 1%, or 1.5% neem oil, 1 g neem leaf powder for consecutive 3 days produced dose dependent pupicidal effect.

Conclusion

Our results indicate that neem products have a good potential to be developed into an effective larvicidal and pupicidal agent for controlling house fly population, especially at their breeding site like poultry houses.

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