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OPERATIONAL NOTE

EVALUATION OF METHYLATED SOY OIL AND WATER-BASED FORMULATIONS OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* AND GOLDEN BEAR OIL® (GB-1111) AGAINST *ANOPHELES QUADRIMACULATUS* LARVAE IN SMALL RICE PLOTS[1,2]

JAMES A. DENNETT,[3] RICHARD L. LAMPMAN,[4] ROBERT J. NOVAK[4] AND MAX V. MEISCH[3]

ABSTRACT. The efficacy of formulations containing methylated soybean oil (MSO) alone and with technical-grade *Bacillus thuringiensis* var. *israelensis* (*Bti*) were compared to Golden Bear Oil® (GB-1111) and a water-based *Bti* formulation against 3rd- to 4th-stage *Anopheles quadrimaculatus* larvae confined to sentinel cages in small rice plots. Three replicates each of MSO with 2% Pyroter® added as a surfactant (MSO + PYR), MSO with 2% Pyroter and 4 g of *Bti* technical powder (MSO + PYR + *Bti*), GB-1111, a water-based formulation with 4 g of *Bti* technical powder (*Bti* + water), and untreated controls were performed. Mosquito larvae were introduced on the 1st day of treatment and at 4 days posttreatment. Mortality was recorded at 24 and 48 h posttreatment for the 1st installation and at 5 days posttreatment for the 2nd installation. The *Bti* + water formulation provided 71% control and the MSO + PYR + *Bti* formulation achieved 64% control, whereas MSO + PYR and GB-1111 produced 16 and 18% control, respectively, at 24 h posttreatment. With the exception of MSO + PYR + *Bti*, which decreased by 2%, the mean percent control increased slightly at 48h posttreatment across remaining treatments, with *Bti* + water obtaining 72% control. This was significantly higher than GB-1111, which achieved 23% control at 48 h posttreatment. The MSO + PYR and MSO + PYR + *Bti* formulations yielded 56 and 62% control, respectively, during the same interval and were not significantly different from one another. Formulations containing MSO + PYR exhibited delayed activity similar to GB-1111, with all formulations except MSO + PYR + *Bti* providing greatest control at 48 h posttreatment. Both MSO formulations (MSO + PYR + *Bti* and MSO + PYR) were statistically comparable to *Bti* + water and GB-1111, respectively, at 24 and 48 h posttreatment. None of the formulations exhibited a residual activity adequate enough to control *An. quadrimaculatus* larvae for up to 5 days.

KEY WORDS *Anopheles quadrimaculatus*, soybean, vegetable oil, *Bacillus thuringiensis* var. *israelensis*, larvicide, GB-1111

In the past, attempts have been made using monomolecular surface films, such as Arosurf®, with *Bacillus thuringiensis* var. *israelensis* de Barjac (*Bti*) or *Bacillus sphaericus* Neide in order to develop formulations with longer residual activity against mosquito larvae and pupae, in addition to repelling ovipositing females (Levy et al. 1984, 1986; Beehler and Mulla 1996). The major drawback to these formulations was that they required vigorous, continuous mixing before application. An Arosurf + *Bti* formulation was found to be highly effective against mixed larval and pupal populations of *Psorophora*, *Aedes*, and *Culex* spp. in roadside ditches, with 100% control reported at 24–48 h posttreatment (Levy et al. 1984). Similarly, Perich et al. (1987) reported that Arosurf combined with *Bti* yielded 90% mortality of all developmental stages, except eggs, of *Anopheles albimanus* Wied. at 48 h posttreatment.

In Florida, Floore et al. (1991) compared the effectiveness of a vegetable oil formulation with *Bti* to a petroleum oil larvicide against *Aedes taeniorhynchus* (Wied.), *Culex quinquefasciatus* Say, and *Culex nigripalpus* Theobald. The vegetable oil formulation (JAXOIL) consisted of 50% VectoBac® 12AS (*Bti*, 1,200 ITU/mg) and 50% recycled vegetable oil (80% soybean, 15% peanut, and 5% cottonseed). In small plot tests, Witco Golden Bear Oil® (GB-1111) applied at 14 liters/ha yielded 100% mortality in all 3 species at 24 h posttreatment. JAXOIL applied at 1 liter/ha produced 95, 98, and 99% mortality, whereas VectoBac 12AS, applied at the same rate, yielded 92, 100, and 95% mortality, respectively, for all 3 species. Although not as good as GB-1111, JAXOIL was slightly better at controlling *Ae. taeniorhynchus* and *Cx. nigripalpus* than VectoBac 12AS, which was more effective in controlling *Cx. quinquefasciatus*. The JAXOIL formulation reportedly provided a stable

[1] Mention of commercial products does not imply a recommendation for use or sale by the University of Arkansas or the Illinois Natural History Survey, Center for Economic Entomology, Entomology Laboratory.

[2] Approved for publication by the Director of the Arkansas Agricultural Experiment Station.

[3] Department of Entomology, University of Arkansas, Fayetteville, AR 72701.

[4] Illinois Natural History Survey, Center for Economic Entomology, Entomology Laboratory, Champaign, IL 61820.

suspension that increased cost effectiveness and total acreage per treatment.

During an evaluation of petroleum-based larvicidal oils and their effects on nontarget organisms, Mulla and Darwazeh (1982) reported that GB-1111 provided excellent control of *Culex tarsalis* Coquillet, *Culiseta inornata* Williston, and *Anopheles franciscanus* McCracken at 7 days posttreatment at applied rates of 18.7 and 37.4 liters/ha. One hundred percent control was obtained 2 days posttreatment at the lower rate for all developmental stages, whereas the higher rate achieved 96%. At 7 days posttreatment, the low rate yielded 67% control compared to the high rate, which maintained 93% control. Although effective control against mosquito larvae was accomplished, detrimental effects were noted on dytiscids, hydrophilids, corixids, and notonectids shortly after treatment.

A recent study showed that methylated soy oil (MSO) mixed with a nonionic surfactant was as effective as GB-1111 in laboratory bioassays against *Culex pipiens* L. and *Anopheles stephensi* Liston (Lampman et al. 2000). Based on this study, a field test was conducted in 1999 to determine the effectiveness of MSO alone and with technical-grade *Bti* powder, GB-1111, and a water-based formulation of *Bti* against *Anopheles quadrimaculatus* Say larvae confined to sentinel cages in treatment plots.

A randomized complete block design was used to test differences between 4 larvicidal formulations from July 8 to 13, 1999, at the Rice Research and Extension Center, Stuttgart, AR. The 15 experimental rice plots measured 7.6 × 7.6 m (25 × 25 ft) and were separated by earthen levees. Each plot was planted with Bengal variety rice, about 0.76 m (30 in.) in height. The plots were maintained with a water depth of 10.2 cm (4 in.) and 20.3 cm (8 in.) in the pan and ditch areas, respectively.

Three replicates were performed with treatments consisting of MSO with 2% Pyroter® (a surfactant) (MSO + PYR), MSO with 2% Pyroter and 4 g of *Bti* technical powder (6,656 ITU/mg, Abbott Laboratories, North Chicago, IL; MSO + PYR + *Bti*), GB-1111 (Golden Bear Oil Specialties, Oildale, CA), a water-based *Bti* formulation with 4 g of *Bti* technical powder (*Bti* + water), and untreated control checks. For MSO + PYR, MSO + PYR + *Bti*, and GB-1111, a volume of 100 ml of each formulation was added to 1,900 ml of water in a 2-liter beverage container, which was agitated and sprayed over the designated plots using a calibrated CO_2 pressurized sprayer until the container was emptied. This procedure was repeated again such that the volume applied to each plot was 4 liters containing 200 ml of active ingredient and 3,800 ml of water. For the *Bti* + water formulation, 4 g of *Bti* technical powder was added to 2 liters of water, agitated, and sprayed over designated plots, repeating the process with another 2 liters of water to clean out the bottle and raise the final volume in these plots to 4 liters as well. The rates applied across all treatments equated to approximately 46.9 liters/ha (5 gal/acre) total volume, with oil formulations approximating 17.3 liters/ha (1.84 gal/acre). Before application, a single sentinel cage was placed within a ditch of each plot. Sentinel cages were of the same design described by Sandoski et al. (1986), with the cages covered with nylon tulle fastened by rubber bands.

Table 1. Mean percent control of *Anopheles quadrimaculatus* larvae in Arkansas small rice plots using methylated soybean oil (MSO) containing the surfactant Pyroter® (MSO + PYR) with and without *Bacillus thuringiensis* var. *israelensis* (*Bti*), Golden Bear larvicidal oil (GB-1111), and water-based *Bti* formulation (*Bti* + water).

Treatment	Installation no.	Reading[1]	Mean percent control[2]
Bti + water	1	24 h	71.1A
MSO + PYR + *Bti*	1	24 h	64.4A
MSO + PYR	1	24 h	15.5B
GB-1111	1	24 h	18.3B
Bti + water	1	48 h	72.4A
MSO + PYR + *Bti*	1	48 h	62.4A
MSO + PYR	1	48 h	55.5AB
GB-1111	1	48 h	22.7B
Bti + water	2	5 days	3.7A
MSO + PYR + *Bti*	2	5 days	3.7A
MSO + PYR	2	5 days	3.3A
GB-1111	2	5 days	13.7A

[1] Twenty-four-hour posttreatment F ratio = 2.77, df 11, $P > F = 0.11$; 48-h posttreatment F ratio = 2.12, df 11, $P > F = 0.17$; 120-h posttreatment F ratio = 0.58, df 11, $P > F = 0.64$.

[2] Mean percent control for specific formulations in a given larval installation on a given reading date followed by the same uppercase letter are not significantly different from one another ($P > 0.10$) using Student's *t*-test.

Anopheles quadrimaculatus larvae used in the test were obtained from cultures maintained at the research station using bloodfed mosquitoes aspirated from livestock barns (Dennett and Meisch, unpublished data). During the test, 2 installations of 10 3rd- to 4th-stage *An. quadrimaculatus* larvae were made in each treatment plot, the 1st installation immediately after treatment on July 8, and the 2nd installation performed 4 days posttreatment on July 12. Observations were made at 24 and 48 h posttreatment for the 1st installation, whereas the 2nd installation was read at 5 days posttreatment to evaluate residual activity.

Field data were corrected for untreated control mortality using Abbott's formula and arcsine transformed before conducting an analysis of variance (Abbott 1925, Sall and Lehman 1996). Mean numbers of dead *An. quadrimaculatus* larvae per treatment were compared using Student's *t*-test.

The *Bti* + water based formulation provided 71% control, whereas the MSO + PYR + *Bti* formulation achieved 64% control at 24 h posttreatment (Table 1). Although not significantly different from one another, both compounds were significantly more active than the MSO + PYR and GB-1111 formulations, which produced 16 and 18% control during the same period, respectively.

Mortalities of the treatments at 48 h were similar to those at 24 h, except for MSO + PYR, which exhibited a 40% increase in mean percent mortality. The *Bti* + water produced the highest mortality, about 72% greater than the control, which was significantly greater than GB-1111, which yielded about 23% control (Table 1). The MSO + PYR and MSO + PYR + *Bti* formulations yielded 56 and 62% control, respectively, and were not significantly different from one another; however, MSO + PYR was also not significantly different from GB-1111 at 48 h posttreatment.

None of the formulations exhibited any long-term residual activity. At 5 days posttreatment, *Bti* + water, MSO + PYR, and MSO + PYR + *Bti* formulations showed about 4% control. Although the activity of GB-1111 was higher at 14% control, its activity was not significantly greater than that of the other treatments (Table 1).

In terms of effectiveness, both *Bti* + water and MSO + PYR + *Bti* were comparable on all 3 dates. In contrast, MSO + PYR and GB-1111 were ineffective at 24 h, whereas MSO + PYR was 2-fold more active than GB-1111 at 48 h posttreatment. All formulations exhibited little efficacy at 5 days posttreatment. Activity of GB-1111 remained low on all observation dates.

Although it is realized that granular formulations would have penetrated the canopy of treatment plots, this study was designed to evaluate MSO as a potential mosquito larvicide under field conditions and to determine whether MSO prolonged the activity of *Bti*. In these tests, GB-1111 achieved 23% control against *An. quadrimaculatus* at 2 days posttreatment at 17.3 liters/ha (1.84 gal/acre), slightly less than the lowest rate used by Mulla and Darwazeh (1982). Although a different species of *Anopheles* was involved, the findings from the present study in terms of extended control were inconsistent with the study by Mulla and Darwazeh at a similar low rate of application. These inconsistencies could be attributed to differences in plot type and number of plots used during both studies. The MSO + PYR formulation provided its greatest control at 48 h posttreatment, which attained a 2-fold higher percent mortality of *An. quadrimaculatus* larvae than with GB-1111, although by 5 days posttreatment, all formulations were statistically equivalent. During the course of these tests, corixid and hydrophilid adults were adversely affected in GB-1111 treatment plots, which was consistent with the findings of Mulla and Darwazeh (1982). No deleterious effects were observed against nontarget species in MSO plots, suggesting that MSO was much safer as a surface larvicide than is GB-1111.

The *Bti* + water and MSO + PYR + *Bti* formulations were not significantly different in these trials; however, mosquito mortality in the *Bti* + water treatments remained in excess of 71% at 24 and 48 h posttreatment, whereas the MSO + PYR + *Bti* formulation achieved about 62% control of mosquitoes during the same period. The MSO + PYR formulation required 48 h before 55% control was attained, whereas only 23% control was provided by the GB-1111. Relatively speaking, *Bti* + water and MSO + PYR + *Bti* produced quick kill of *An. quadrimaculatus* larvae, compared to the non-*Bti* formulations. Calculated *Bti* potency was extremely high, because both the *Bti* + water and MSO + PYR + *Bti* formulations contained approximately 13,312 ITU/ml, or 2 mg *Bti* technical powder/ml in the 1st 2-liter bottles used. Even at these concentrations, larvicidal activity was rather short-lived in the rice habitat.

Although the control produced by the MSO + PYR + *Bti* formulation was not significantly different from that of the *Bti* + water, the MSO + PYR formulation produced better control than did GB-1111 at 48 h posttreatment. Although none of the formulations achieved a level of control necessary for mosquito abatement in rice habitats, further research is needed to determine if extended residual activity could be gained against *An. quadrimaculatus* larvae using vegetable oil–based larvicides containing *B. sphaericus*.

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REFERENCES CITED

Abbott WS. 1925. A method for computing the effectiveness of an insecticide. *J Econ Entomol* 18:265–267.

Beehler JW, Mulla MS. 1996. Larvicidal oils modify the oviposition behavior of *Culex* mosquitoes. *J Vector Ecol* 21:60–65.

Floore TG, Clements BW Jr, Dukes JC, Simmonds PR, Boike AH Jr, Greer MG, Coughlin JC. 1991. Evaluation of a Vectobac-12AS/vegetable oil formulation for the control of *Aedes taeniorhynchus, Culex quinquefasciatus,* and *Culex nigripalpus* larvae compared with Witco Golden Bear (GB-1111). *J Fla Mosq Control Assoc* 62(2):41–44.

Lampman R, Eckenbach U, Seigler D, Novak R. 2000. Laboratory evaluations of methylated soy oil and monoterpenes as mosquito larvicides. *J Am Mosq Control Assoc* 16:153–157.

Levy R, Powell CM, Hertlein BC, Miller TW Jr. 1984. Efficacy of Arosurf® MSF (monomolecular surface film) base formulations of *Bacillus thuringiensis* var. *israelensis* against mixed populations of mosquito larvae and pupae: bioassay and preliminary field evaluations. *Mosq News* 44:537–543.

Levy R, Putnam JL, Miller TW Jr. 1986. Laboratory evaluations of formulations of Arosurf® MSF and *Bacillus sphaericus* against larvae and pupae of *Culex quinquefasciatus. J Am Mosq Control Assoc* 2:233–236.

Mulla MS, Darwazeh HA. 1982. Efficacy of petroleum larvicidal oils and their impact on some aquatic nontarget organisms. *Proc Pap Calif Mosq Control Assoc* 49:84–87.

Perich MJ, Rogers JT, Boobar LR. 1987. Efficacy of Arosurf® MSF and formulations of *Bacillus thuringiensis* var. *israelensis* against *Anopheles albimanus*: laboratory bioassay. *J Am Mosq Control Assoc* 3:485–488.

Sall J, Lehman A. 1996. *JMP® start statistics: a guide to statistics and data analysis using JMP and JMP IN® software* Cary, NC: SAS Institute.

Sandoski CA, Yearian WC, Meisch MV. 1986. Swath width determination for Beecomist®-applied *Bacillus thuringiensis* (H-14) against *Anopheles quadrimaculatus* larvae in rice fields. *J Am Mosq Control Assoc* 2:461–468.