Stabilization of Azadirachtin A in Neem Formulations: Effect of Some Solid Carriers, Neem Oil, and Stabilizers

Jitendra Kumar and Balraj S. Parmar*

Division of Agricultural Chemicals, Indian Agricultural Research Institute, New Delhi 110012, India

Formulation of azadirachtin A on attapulgite, kaolinite, fuller's earth, hydrated calcium silicate, and fly ash revealed that it degraded to the tune of 70−95% on different solid carriers as compared to 56% in neem oil, during the 14 day heat storage studies at 54 ± 1 °C in the laboratory. The degradation was reduced by 26−60% on different carriers by employing either anthraquinone or epichlorohydrin as stabilizer. Pyrogallol and hydroquinone enhanced the degradation. The cation exchange capacity and surface area of the carriers revealed a significant negative correlation with $t_{1/2}$ of azadirachtin A.

Keywords: *Azadirachtin A; solid pesticide carriers; neem oil; stabilizers; powders*

INTRODUCTION

Azadirachtin A is the currently accepted reference ingredient for standardizing neem-based products (GTZ-UNIDO-RENPAP, 1996). However, its lability to heat, moisture, air, etc. (Stokes and Redfern, 1982; Barnby et al., 1989) has been a matter of concern that led to global efforts to stabilize it (Raguraman and Jayaraj, 1994; Stark and Walter, 1995; Sundaram, 1996; Chowdhury, 1996). At present, only an emulsifiable concentrate formulation of neem is extensively used all over the world. Azadirachtin A is usually stabilized by adding epichlorohydrin. Scant information is available on the development of solid formulations based on neem materials (Parmar and Srivastava, 1986).

The interaction between clay carriers and active ingredients is well documented (Parmar and Dureja, 1994). The stability and performance of the active ingredients vary with the chemical, formulation auxiliaries, environmental, and other factors (Dragon, 1988; Parmar and Dureja, 1994). Therefore, it was considered of interest to explore the development of azadirachtin A based powder formulations employing different carriers. Some of the well-known phenolic (pyrogallol), quinone (anthraquinone, hydroquinone), and alkoxy (epichlorohydrin) derivatives (Sayed et al., 1974) have been evaluated as stabilizers for azadirachtin A.

MATERIALS AND METHODS

Test Carriers. Commercial grade attapulgite, kaolinite, and fuller's earth (MCA Industries, New Delhi, India) and hydrated calcium silicate (courtesy of Hindustan Insecticides Ltd., New Delhi, India) were procured in 1986 and were available in the Division. Their physicochemical properties were reported earlier (Rengasamy and Parmar, 1988). Fly ash (pH in 2 times water, 7.25) was obtained from Indraprastha thermal power station, New Delhi, India. The specific surface areas ($m^2\ g^{-1}$) of the test carriers as determined by the monoethylene glycol absorption method (Carter et al., 1965) were as follows: attapulgite, 116.8; kaolinite, 34.1; fuller's earth, 510, fly ash, 146.0; hydrated calcium silicate, 726.1. Their cation exchange capacities (mequiv 100 g^{-1}) as determined by Schollenberger's ammonium acetate method (Piper, 1950) were as follows: attapulgite, 17.90; kaolinite, 24.02; fuller's earth, 96.24; hydrated calcium silicate, 205.5; and fly ash, 10.0.

Stabilizers. Anthraquinone (**1**), pyrogallol (**2**), hydroquinone (**3**), and epichlorohydrin (**4**) were locally procured.

Azadirachtin A. Reference azadirachtin A (purity = 95%, HPLC, Trifolio-M GMBH, Germany) was obtained through the courtesty of Neem Mission, Pune, India. Technical aza-A (purity = 25%, HPLC) was available. It was used for preparing different powder formulations.

Solvents and Chemicals. For routine laboratory work, laboratory grade, and for HPLC analysis, analytical grade, chemicals and/or solvents were employed.

Preparation of Dusts. Dusts were prepared on different carriers with or without the stabilizers. One gram of each carrier was impregnated with the solution of technical aza-A in acetone to yield 2540 ppm aza-A dust. In stabilizer treatments, an acetone solution of the stabilizer was added to the impregnated dust to obtain a 0.5% stabilizer level (dust basis). Acetone was evaporated at room temperature (30 °C) by frequently agitating the content with a glass rod. The dried masses were blended in a laboratory model planetary ball mill (Fritsch Pulverisette, Germany). To evaluate the effect of stabilizer, the dusts impregnated with aza-A alone served as control.

Azadirachtin A Enriched Neem Oil. The oil was extracted from neem kernel according to the method of Kumar and Parmar (1996). It was enriched with technical aza-A (required quantity added in MeOH) to prepare a 1000 ppm aza-A oil sample.

Three grams each of neem oil sample was prepared at room temperature with or without the stabilizer (0.5% oil basis). Aza-A enriched oil samples served as control in the evaluation of the effect of the stabilizers.

Extraction of Aza-A from Powders. Aza-A was extracted from the periodically withdrawn 0.1 g dust samples in 65:35 aqueous methanol. The samples were magnetically stirred for 15 min in conical flasks employing a Teflon stirring paddle, using 5 mL of aqueous methanol. The supernatant liquid was filtered through a Whatman No. 42 filter paper. The residue was re-extracted in aqueous methanol (2 × 2.5 mL) and filtered. The filtrates were combined, and the final volume was made upto 10.0 mL. The efficiency of extraction was judged by determining the recovery of aza-A from the technical azadirachtin A spiked carriers. Overall mean recoveries on attapulgite, kaolinite, fuller's earth, and fly ash, each spiked

* Author to whom correspondence should be addressed (fax 011-91-11-576-6420; e-mail guest@bic.iari.ren.nic.in).

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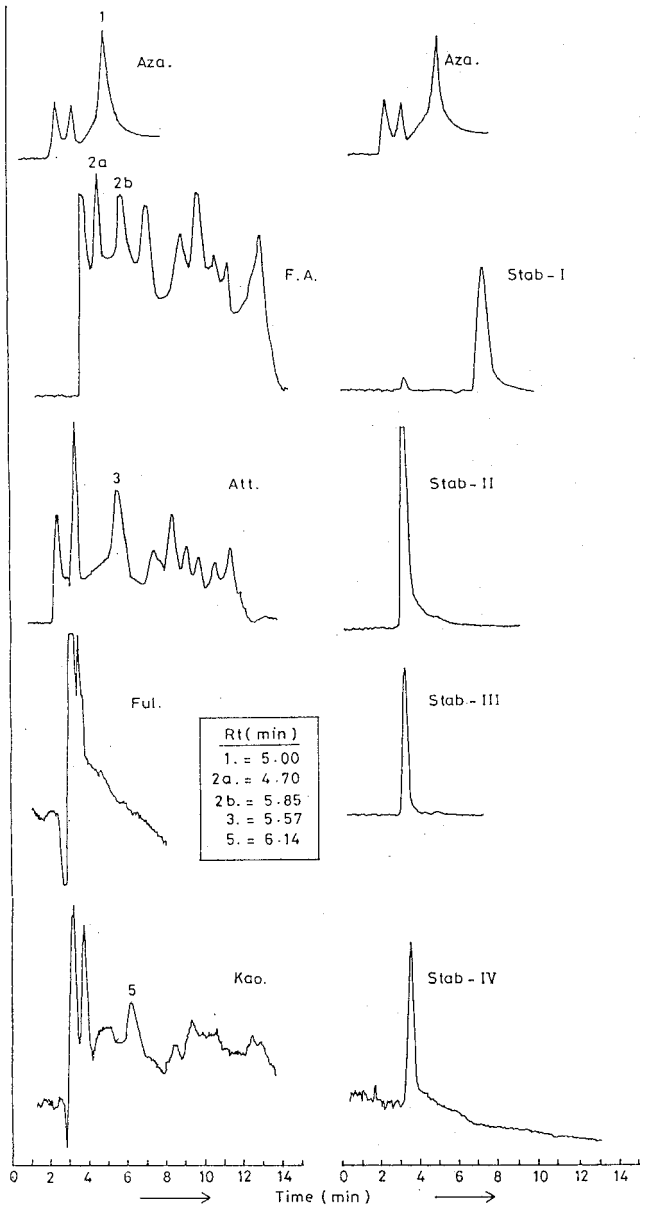

Figure 1. HPLC chromatogram of carriers, stabilizers, and azadirachtin A.

with 300 ppm of aza-A, were 81.5, 80.0, 78.0, and 75.0%, respectively.

Extraction of Aza-A from Neem Oils. Aza-A in 0.5 g neem oil samples was extracted according to the method of Kumar and Parmar (1996).

Evaluation of Stability of Aza-A. Time for loss of 50% aza-A ($t_{1/2}$) on different solid carriers as well as in neem oil was determined in triplicate in heat storage studies at 54 ± 1 °C for 14 days. The samples were drawn at 0, 1, 3, 7, and 14 days of incubation.

HPLC Analysis. Aza-A in aqueous methanol was analyzed by employing a Shimadzu HPLC fitted with LC9A pumps, a 20 μL loop, and a SPDM6A photodiode array detector. Samples were resolved isocratically on a 15 cm × 6 mm i.d. Shimpack CLC phenyl stainless steel column using methanol/water (65:35) mobile phas at 1.0 mL min^{-1}. Each chromatogram was run for up to 20 min. The absorbance was measured at 214 and 250 nm at a sensitivity of 0.05 AUFS. The data were acquired on a PCS-DG India Ltd. workstation, and quantification was done in the postanalysis session. Aza-A showed a retention time of 5.00 min with a limit of detection of ~25 ppm on carriers. Some peaks in extracts of fly ash (4.70 and 5.85), attapulgite (5.57), and kaolinite (6.14) showed retention times

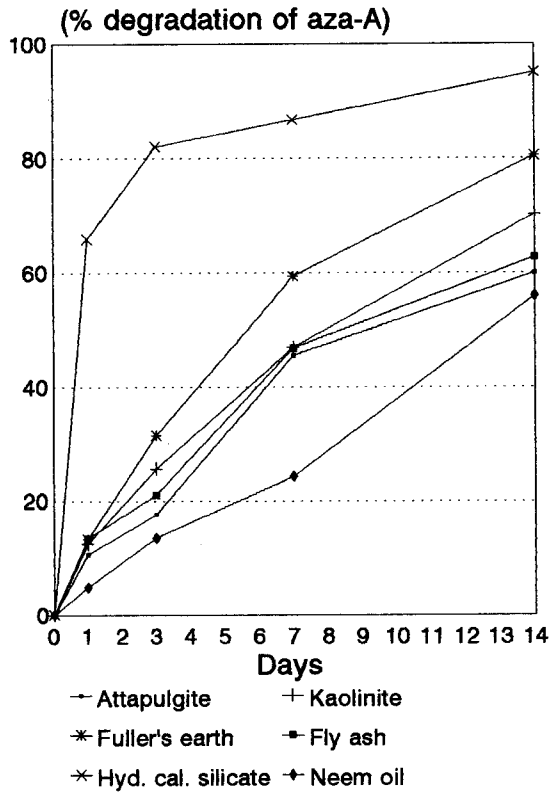

Figure 2. Degradation of azadirachtin A on different carriers.

close to that of aza-A. None of these, however, interfered with its quantification (Figure 1). The carry-over peaks were observed during analysis and were eliminated by flushing the HPLC column with methanol/water (65:35).

Data Analysis. The aza-A content obtained from each treatment with time was fitted into the first-order rate equation $A = A_0\, e^{-ct}$, and $t_{1/2}$ values and regression coefficient (slope) were computed using the software package Microstat.

$$t_{1/2} = -\ln(A/A_0)/C = (\ln 2)/C$$

A is the aza-A concentration at time t, A_0 is the initial concentration, and C is the rate constant. The data were subjected to analysis of covariance, taking time as covariate, separately for carriers and stabilizers using the GLM procedure of the SAS package. Because the design effect is taken as considered in the analysis of covariance, the individual regression coefficients are not estimable. The regression coefficients obtained using analysis of covariance in SAS being biased were not useful for any interpretation except for testing the significance of stabilizers and carriers. The slopes as obtained by fitting aza-A content on time have been reported in Table 1.

RESULTS AND DISCUSSION

Effect of Carrier on Degradation of Aza-A. The rate of degradation of aza-A on different solid carriers and in neem oil in the laboratory incubation studies is shown in Figure 2. After 14 days of incubation, the degradation was the fastest on hydrated calcium silicate (95%) followed by fuller's earth (80.5%). It was the slowest on attapulgite (60%). In neem oil 56% degradation was observed. Relatively lower loss of aza-A in neem oil and on attapulgite may be attributed to a favorable interaction with these materials. The relative degradation of aza-A on different carriers followed the order hydrated calcium silicate (95%) > fuller's earth (80.5%) > kaolinite (70.2%) > fly ash (62.7%) > atta-

Table 1. Half-Life ($t_{1/2}$, Days) of Azadirachtin A on Different Carriers with and without Different Stabilizers

	stabilizer									
	control		anthraquinone		epichlorohydrin		hydroquinone		pyrogallol	
carrier	$t_{1/2}$	slope	$t_{1/2}$	slope	$t_{1/2}$	slope	$t_{1/2}$	slope	$t_{1/2}$	slope
attapulgite	10.59	0.0284	20.47	0.0147	16.90	0.0178	5.37	0.0561	3.63	0.0830
kaolinite	8.13	0.0370	11.36	0.0265	11.66	0.0258	4.89	0.0615	4.20	0.0712
fuller's earth	5.88	0.0513	7.64	0.0394	7.05	0.0423	4.98	0.0604	3.67	0.0819
fly ash	9.78	0.0308	16.25	0.0185	14.12	0.0213	5.48	0.0549	3.69	0.0816
neem oil	12.24	0.0246	26.18	0.0115	15.92	0.0189	7.50	0.0401	4.83	0.0623
hydrated calcium silicate[a]	3.78	0.0796								

Analysis of Covariance Using Time as Covariate

source	DF	type 1 SS	mean square	*F* value	Pr > *F*
carrier	4	28.1538	7.0385	438.76	0.0001
stabilizer	4	8.6171	2.1543	134.29	0.0001
carrier × stabilizer	16	0.5053	0.0316	1.97	0.0145
time	1	17.5549	17.5549	1094.34	0.0001
time × carrier	4	0.5683	0.1421	8.86	0.0001
time × stabilizer	4	3.9989	0.9997	163.95	0.0001

[a] Not included for statistical analysis.

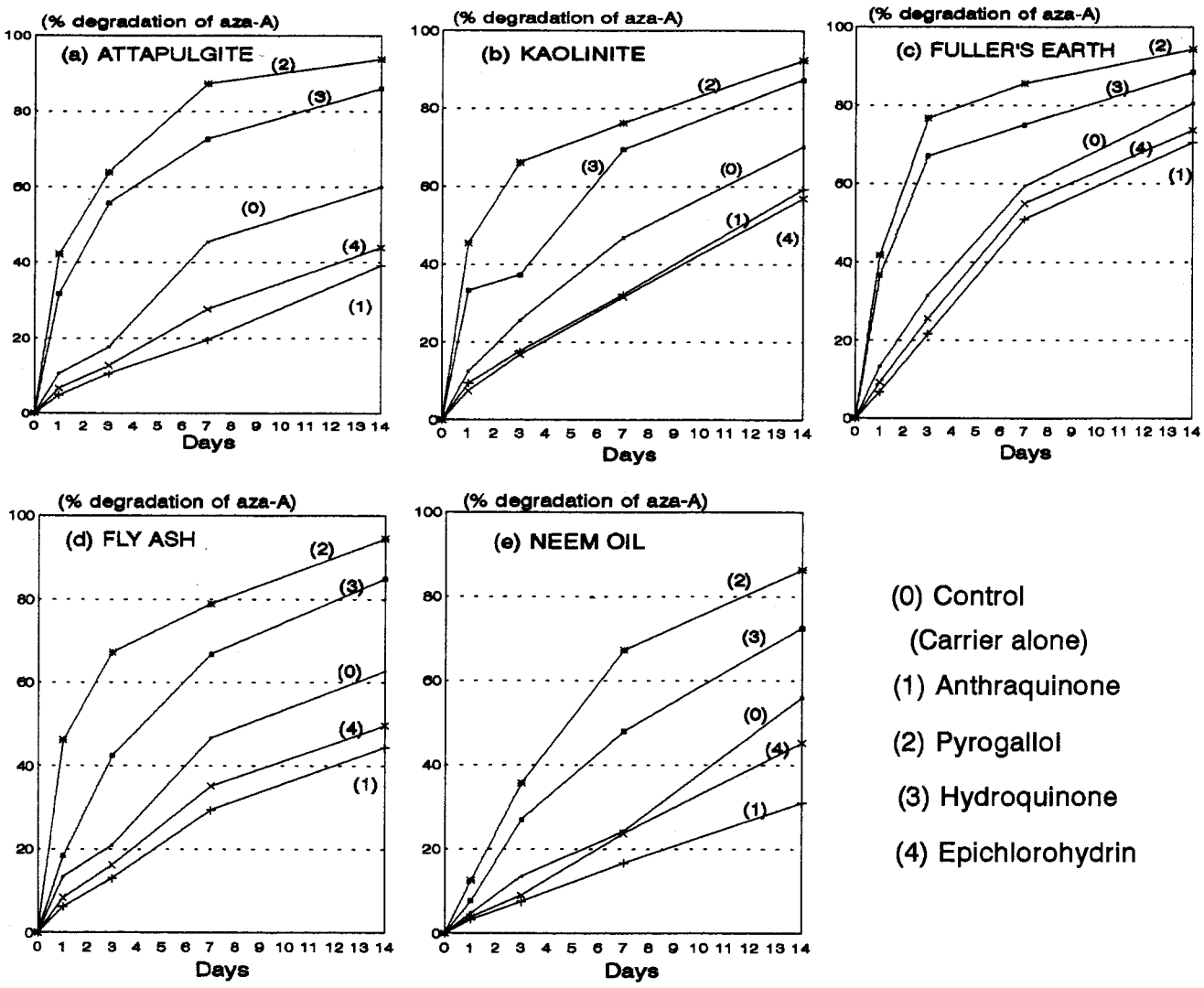

Figure 3. Degradation of azadirachtin A on different carriers along with different stabilizers.

pulgite (60.0%) > neem oil (56.0%).

The $t_{1/2}$ showed a significant correlation with the CEC ($r^2 = -0.94$) and the surface area ($r^2 = -0.90$) of the carriers. Kaolinite showed a higher degradation than attapulgite even though its surface area was less than its. Perhaps its other constituents are important in degrading aza-A.

In view of the instant high degradation of aza-A on hydrated calcium silicate, its stabilization on this carrier was not attempted.

Effect of Stabilizers in Checking Degradation of Aza-A. The relative orders of aza-A stabilization by different stabilizers on different carriers on the 14th day of incubation (Figure 3) were as follows: attapulgite,

anthraquinone > epichlorohydrin > control > hydroquinone > pyrogallol; kaolinite, epichlorohydrin > anthraquinone > control > hydroquinone > pyrogallol; fuller's earth, anthraquinone > epichlorohydrin > control > hydroquinone > pyrogallol; fly ash, anthraquinone > epichlorohydrin > control > hydroquinone > pyrogallol; neem oil, anthraquinone > epichlorohydrin > control > hydroquinone > pyrogallol.

The trend of aza-A stabilization by different stabilizers was the same on different carriers except kaolinite. Maximum stabilization of aza-A was recorded with anthraquinone followed by epichlorohydrin. Pyrogallol and hydroquinone, the phenolic antioxidants, promoted aza-A degradation instead of its stabilization. Additional research is necessary to understand the structure-effect relationship and degradation/stabilization of the aza-A molecule by different molecules of stabilizers.

Anthraquinone (Stabilizer 1). Except on kaolinite, anthraquinone was the most effective aza-A stabilizer on all of the carriers (Figure 3). The degradation of aza-A on the 14th day was reduced by 29.4−69%, the maximum reduction being in neem oil (69%) followed by attapulgite (60.8%), fly ash (55.5%), kaolinite (40.7%), and fuller's earth (29.4%) (Figure 4a). In an earlier study, 2,4-dihydroxybenzophenone has been reported to reduce the photodegradation of aza-A (Sundaram, 1996). Anthraquinone seems to be playing a similar role in preventing photo-/thermo-/hydrolytic or any other type of degradation of aza-A.

Pyrogallol (Stabilizer 2). Addition of pyrogallol to aza-A formulations increased its rate of degradation on all carriers (Figures 3 and 4). By the 3rd day of incubation, >64% of aza-A was degraded on all of the carriers except neem oil (35.7%). By the 14th day of incubation 86.3−94.5% of aza-A was degraded on all of the carriers.

Hydroquinone (Stabilizer 3). Hydroquinone behaved in a similar fashion to pyrogallol with respect to aza-A degradation on different carriers (Figures 3 and 4). However, the rate of degradation of aza-A was slower than in the case of pyrogallol. It was in the range of 72.6−88.4% on the 14th day.

Epichlorohydrin (Stabilizer 4). The trend of stabilization by addition of epichlorohydrin on different carriers is shown in Figures 3 and 4. Maximum stabilization as based on reduced loss of aza-A on the 14th day was observed on attapulgite (56%) and neem oil (54.7%), followed by fly ash (50.3%). Minimum aza-A stabilization was observed in fuller's earth (26.3%).

The addition of anthraquinone and epichlorohydrin thus stabilized aza-A on all of the carriers and in neem oil, whereas pyrogallol and hydroquinone induced its degradation in all cases.

Rate of Degradation. Regression coefficient (slope) and half-life ($t_{1/2}$) of aza-A on different carriers with and without the test stabilizers are given in Table 1. The half-life ranged from 3.63 to 26.18 days. On solid carrier alone (control), it varied from 3.78 to 10.59 days. This variation highlights the variable interactive forces on different carriers. The lowest $t_{1/2}$ values were obtained with pyrogallol and hydroquinone treatments. Analysis of the data employing the SAS GLM procedure revealed that the carriers as well as the stabilizers influenced significantly ($P < 0.0001$) the degradation of aza-A in various treatments. Anthraquinone on attapulgite and in neem oil stabilized aza-A the most. The $t_{1/2}$ of aza-A in these treatments increased by nearly 2 times as

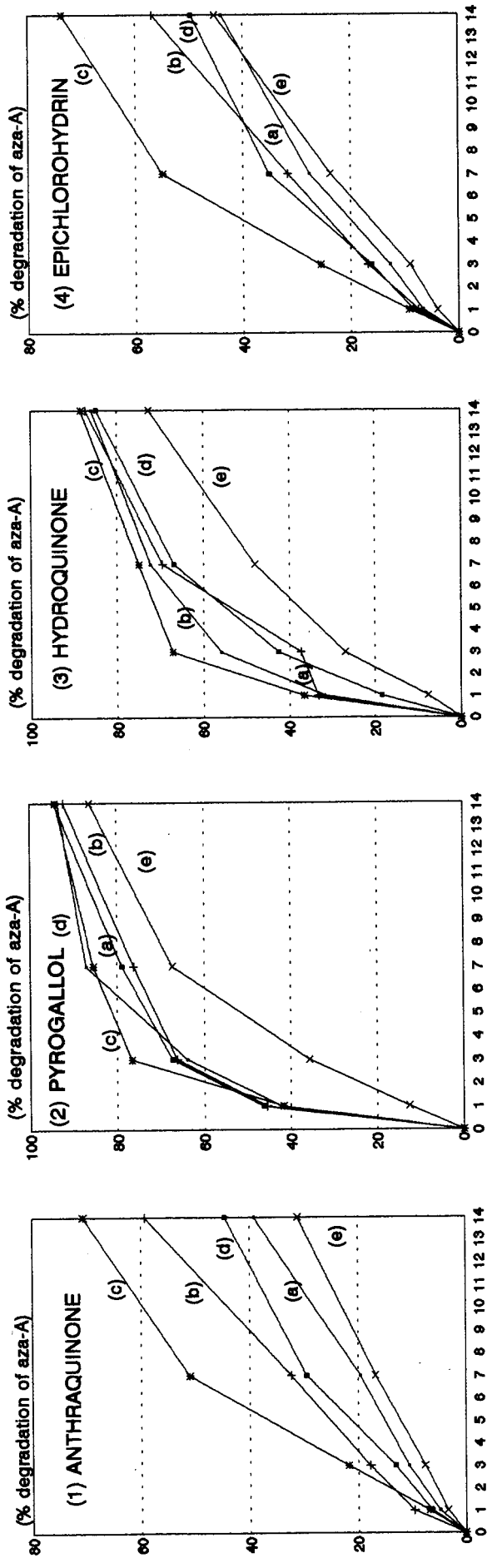

Figure 4. Relative order among carriers with respect to azadirachtin A degradation in the presence of different stabilizers: (a) attapulgite; (b) kaolinite; (c) fuller's earth; (d) fly ash; (e) neem oil.

compared to control. Treatment of aza-A with epichlorohydrin on attapulgite and fly ash increased the half-life by 1.5 times and with neem oil by 1.25 times. Pyrogallol and hydroquinone, two known strong antioxidants, accelerated its degradation instead of stabilizing it. The mechanisms underlying the preferential degradation of aza-A with these antioxidants needs further investigation.

The present study indicates that using anthraquinone and epichlorohydrin in clay based powders or neem oil based products will improve the shelf life of aza-A in these formulations.

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