EFFECT OF SEED EXTRACTS OF *AZADIRACHTA INDICA* A. JUSS ON THE TRANSMISSION OF COWPEA APHID-BORNE MOSAIC VIRUS BY *MYZUS PERSICAE* SUIZ (HEMIPTERA; APHIDIDAE)

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ABSTRACT

Neem seed extracts, at 20, 40, 60, 80, 100 and 140 seed gram per litre distilled water, were directly toxic to *Myzus persicae*. But the lower the storage temperature or the higher the concentration of extract, then the greater the insecticidal activity of the extract. Water extract was superior to acetone, hexane, methanolic, ethanolic, butanolic, chloroform and petroleum ether extracts in that order. Whereas 20 and 40 g/l acqeuos extracts only showed mild toxicity to the aphids, 100 percent mortality was induced by 100, 120, and 140g/l water extracts. Besides, storing the extract at 40-80°C reduces the potency of neem extracts. The aphids avoided neem-treated surfaces both *in vitro* and in pot cultures. This established the aphid-repellence property of neem seed extracts. Plants treated with 80, 100, 120 and 140 g/l water extracts produced significantly higher yields ($P<0.05$) than those in pots receiving 20, 40, and 60 g/l extracts.

Keywords: ***Azadirachta indica, Myzus persicae*, Cowpea, inhibition, virus transmission**

INTRODUCTION

Losses of various dimensions due to damage by insect pest on agricultural crops occur every year. Although chemical pesticides are known to be very effective in pest control (Gruzdyev *et al.*, 1988), their prohibitive costs and toxic side effects have stimulated considerable interest in botanical pest control substances especially in Nigeria where over 98 percent of the food consume is produced by peasant farmers, ,who are least knowledgeable in the use of chemical pesticides.

One of the most important flora so far studied for pesticidal properties is the neem (*Azadirachta indica* A. Juss; Meliaceae), which is widely distributed in Africa, Asia and the Americas (Utz, 1993). It was first reported by Chopra, (1928) that the neem tree contains insecticidal principles. Since then investigations on the effect of neem extracts on insects have gained great momentum (Schmutterer *et al.*, 1981; Latum, 1985; Schmutterer and Ascher, 1987; Kleeberg, 1993). Although all parts of the neem tree are known to harbour insecticidal principles, extracts from the seed have been found most toxic to a wide range of insect pest (Schmutterer, 1985, 1990). This is because the neem seed contains the highest concentration of azadirachtin and other biologically active substances (Ermel *et al.*, 1987; Schmutterer, 1988).

While there is ample information on the insecticidal activity of neem products, only very scanty detailed studies focussing on their effects on the virus-insect vector relationship is available (Saxena, 1987). Simons, (1981) emphasized the use of neem oil formulations with antifeedants for control of insect-transmitted virus disease. Neem oil and neem seed extractive were highly effective in reducing the survival of *Nephotettix virescens* and its transmission of rice tungro virus (Mariappan and Saxena, 1983) and ditto to *Nilaparvata lugens* which transmits both grassy stunt and ragged stunt viruses (Saxena and Khan, 1985).

This communication examines the effect of neem seed extracts on the efficiency of green-peach aphid (*Myzus persicae Sulz*; Hemiptera, Aphididae) in transmitting Cowpea aphid-borne mosaic virus. The virus, which induces as severed mosaic or interrenal chlorosis, distortion and stunting on leaves of infected Cowpea plant, is transmitted in a non-persistent manner by the aphid vector (Bock, 1973; CAB/AAB, 1974). Grain yield reductions of up to 87 per cent may be achieved if unchecked (Singh and Allen, 1979).

MATERIALS AND METHODS

Materials:

Seeds obtained from air-dried and decorticated neem fruits were pulverished using ceramic mortar and pestle. The powder was suspended in distilled water and magnetically stirred for 18hr. It was thereafter centrifuge at 5,000 rpm for 5 min and then expressed through four-fold-thick muslin cloth. Aqueous filtrate obtained was prepared in concentrations of 20, 40, 60, 80, 100, 120 and 140 gram of seed per litre distilled water. To obtain organic solvent extracts, 200g of the powdered sample was extracted with 200ml each of acetone, n-Butanol, Chloroform, 95% ethanol, n-hexane, 80% methanol, and petroleum ether using soxhlet extraction technique (Feuerhake, 1985). All extracts were sterilized by passing them through 0.22 or 0.45um (Pore size millipore membrane filer equipped with a swinney filter adaptor.

Ife Brown cultivar of cowpea (*Vigna unguiculate* L. Walp; Fabaceae), on which IT-6 isolate of Cowpea aphid-borne mosaic virus was maintained by regular transfers, served as the test plant. The initial culture of *Myzus persicae* was procured from the Entomology Unit of the International Institute of Tropical Agriculture, Ibadan, Nigeria, and a permanent colony of the aphid was maintained on healthy egg plant (*Solanum melongena* L.; Solanaceae) which is a non-host of the virus (Lapipo, 1976). The fresh cultures of adults of uniform age were kept as the source of non-viruliferous aphids throughout the transmission experiments.

Laboratory Trials

This trial is basically of two types: (a) Freshly harvested leaves of eggplant were painted with the extracts (5ml/leaf) on both sides using a camel hair brush and placed in each sterile petri dish. Apterus adult aphids were starved for 3 hr and then immediately transferred onto the treated leaves (30 aphids per plate). Water soaked leaves and starved aphids serves as the control. (b) 2ul of each extract was spotted on each of the adult aphids in sterile petri dish and compared with a distilled water control. There were thirty aphids per plate. The experiment, in 10 replications, was randomly arranged on laboratory benches at 27 $\pm$ 1°C for 48 hr and repeated two times. Thereafter, percentage mortality over control was determined according to Sombastiri and Temboonkeat, (1987). Percentage mortality was corrected using Abbolt's formula (Abbolt, 1925) and LC 50 was determined.

Effect of Temperature on the Activity of Aqueous Extract:

100g/L aqueous extract was used in this trial because it represents the minimal inhibitory concentration (MIC). 10ml of the extracts in each of clean Bhijo bottles was subjected to different temperature regimes of 20, 30, 40, 50, 60, 70 and 80°C in a water bath for one hour and then left to cool for another one hour before they were tested for infectivity *in vitro*.

Greenhouse tests:

Cowpea test crops raised in 2-litre pots containing sterilized soil were kept in insect - proof cases. Ten-day-old seedlings were painted with 15ml per stand of each extract. Thereafter, the treated plants were inoculated with the virus using apterus adult aphids as described by Simons, (1981). The untreated but inoculated plants served as control. There were ten replicates per treatment in a completely randomized design. Experimental plants were surveyed for symptoms according to Ladipo, (1976). Other parameters measured included plant height, leaf number, grain yield, root and shoot dry weights.

RESULT AND DISCUSSION

Neem seed extracts have shown obvious toxicity to *Myzus persicae*. But such toxicity varied with the extract concentration, the medium of extraction and/or the temperature to which extract is exposed. Generally the higher the concentration (from 20 to 140/L) and/or the lower the temperature (20 - 30°C at which extract is kept before use) then the higher the potency of the neem extract (Figures 1 and 3). The calculated leathal concentration for 50 percent of the aphid biotests (LC_{50}) was 52.3g/L aqueous extract.

The Solvent used in the extraction of the neem seeds also determines the efficacy. In the present study, it has been established that aqueous extract was superior to the organic solvents in inhibiting the survival of *M. persicae in vitro* (Fig. 2). This means that water is a good solvent for the active principles in neem seed as earlier suggested by Feurerhake, (1985). One of the possible explanations for this observation may be the occurrence of a chemical change during the soxhlet extraction procedure i.e. the organic solvents may have reacted with the active ingredients in the neem sample. Water extract was respectively followed in a decreasing order of potency by acetone, hexane, methanolic, butanolic, chloroform and petrol ether extracts at 100g/L. Although the activity of acetone extract did not differ at 5% level, from those of hexane and methanolic treatments. In addition, the activity of ethanolic, butanolic and chloroform treatments was statistically the same (P = 0.05), even though they respectively induced 75%, 65% and 60% aphid mortality (Fig. 2). Petroleum ether extract showed mild toxicity, producing only 18 percent mortality. The observed mortality may have resulted from ingestion of the extracts (Jackai, 1993), or it may be that the extracts interfered with the physiological environment to the detriment of the test organisms (Schmutterer, 1988). Besides, microscopic examination of the treated aphids after 48hrs showed the presence of *Aspergillus flavus* on the dead aphids. The fungus develops and gradually spreads over the dead aphid biotests. There is the need to examine the direct effect of aflatoxin on *Myzus persicae*, since *A. flavus* is the aflatoxin producing fungus in neem seed (Siniah *et al.*, 1983).

When its toxicity was assessed relative to varying temperature regimes, 100g/L aqueous extract still induced 100 percent mortality at 20° and 30°C. But further increase in temperature up till 80°C reduced the activity of the extract (Fig. 3). The effect of increasing temperature on the toxicity of neem seed extract to *M. persicae* is congruent with a pervious

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experiment (Ermel *et al.*, 1987), in which incubation of neem seed kernels under increasing temperature (up to 60°C) resulted in a time - dependent decrease of the content as well as the potency of azadiractin and other biologically active substances in neem seed kernel extract.

Moreover, the aphids avoided neem - treated surfaces both *in vitro* and in pot cultures. The aphid repellency property of the need seed extract (Roa and Pramer, 1984; Saxena *et al.*, 1984; Saxena, 1987) most probably kept the insects away from the treated plant parts (in pot), thereby rendering, the plants unattractive to the insects (Schmutterer, 1990; Ishida *et al.*, 1992). The yields of Cowpea plants receiving 20g/L and 40g/L water extracted did not differ significantly from each other (20 and 25 seed g/plant respectively) and from those of the untreated inoculated pots (21 seed g/plant). Whereas, plants which were treated with 100, 120 and 140g/l extracts gave the best grain yields of 36, 39, and 40 seed g/plant respectively (Table 1).

The results outlined here have shown that, the use of simple, crude botanicals, such as neem seed extracts is ideally suited for crop protection by resource - limited farmers in developing countries. Neem seed can be processed at village level and crude fractions can be extracted using simple methods.

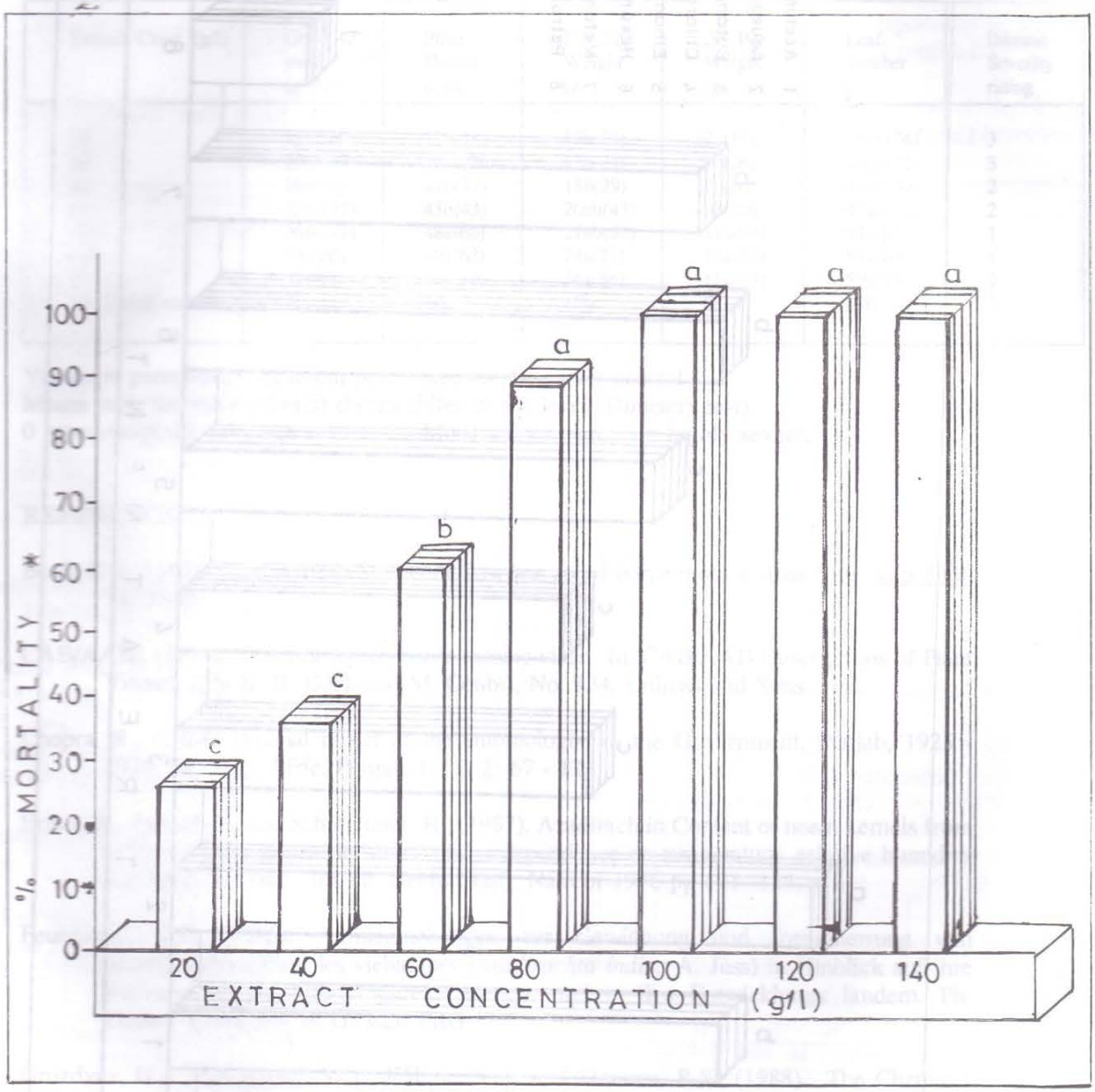

Figure 1: **Percentage mortality of *Myzus persicae* in different concentrations of neem seed extract.**

***Peaks with the same letter do not differ significantly (p = 0.05) using Duncan's test.**

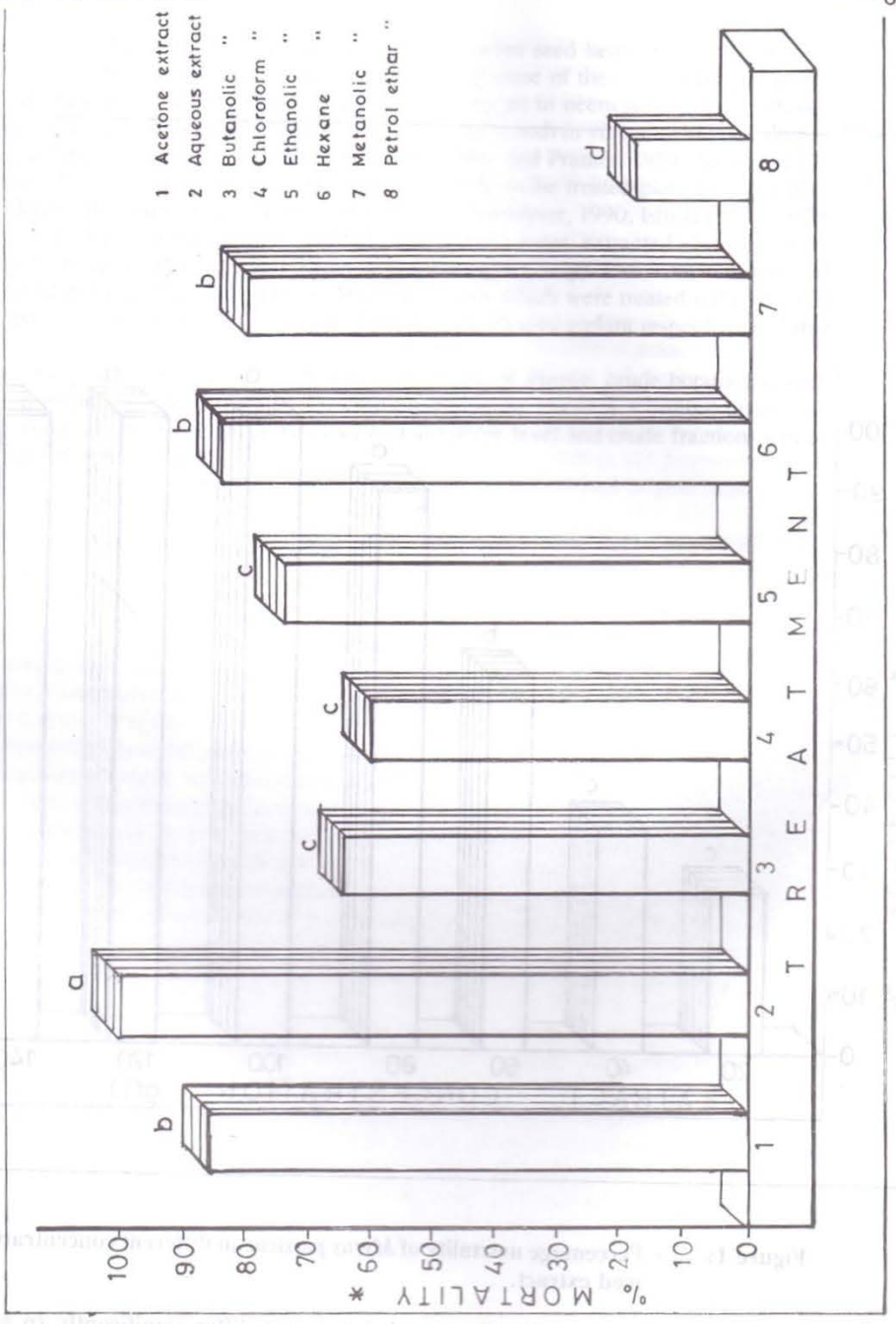

Figure 2: Percentage mortality of *Myzus perciae* in different formulations of neem seed.

*Peaks with the same letter do not differ significantly (p = 0.05) using Duncan's test.

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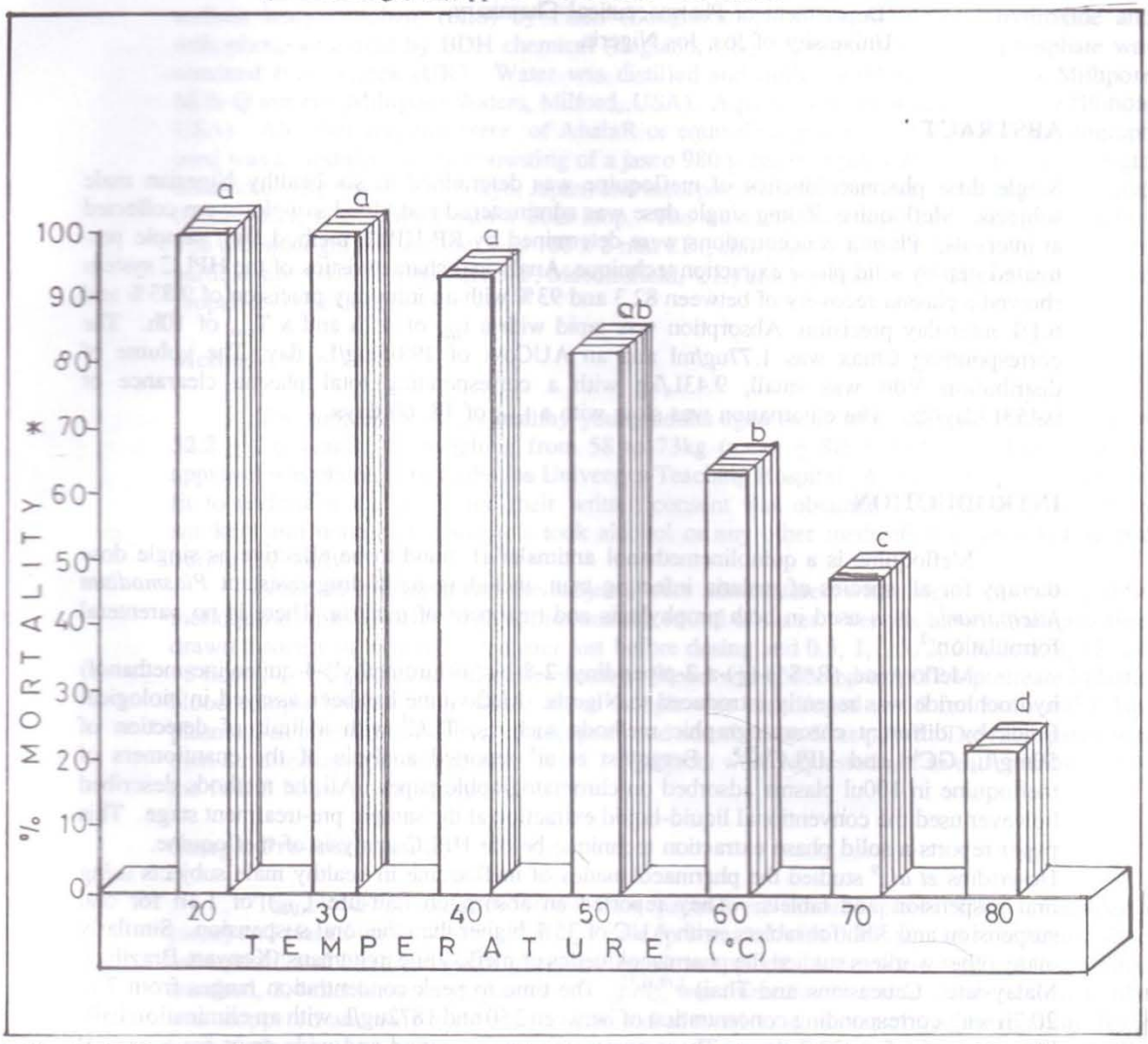

FIG. 3. Effect of temperature on the insecticidal activity of 100g/l aqueous neem seed extract.

* peaks with the same letter(s) do not differ significantly (p= 0.05) using Ducans test.