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(56) Related Art

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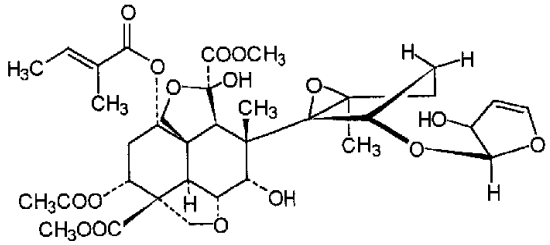
Pesticidal Dry Powder Formulation

Abstract

The invention relates to a preparation of azadirachtin,



in a dry solid powder form having a purity up to 88%; an emulsion concentrate having up to 30% by weight of azadirachtin, and a process for preparing said azadirachtin dry powder from neem seeds/kernels, which comprising: (a) disintegrating the neem seeds/kernels into a powder; (b) subjecting the said powder to continuous extraction using methanol or aqueous methanol or ethanol (rectified spirit) or aqueous ethanol at ambient temperature; (c) concentrating the extract and stirring the concentrate with petroleum ether or hexane and phase separating by conventional methods; (d) stirring the denser phase containing major quantity of azadirachtin with a water immiscible organic solvent and water as required depending on the solvent used for extraction and phase separating by conventional methods; (e) concentrating the organic phase and gradually adding the concentrate to petroleum ether or hexane under stirring at ambient temperature (f), filtering under suction and drying under vacuum at a temperature in the range of 25-65°C. to obtain a neem seed/kernel extract as a powder having azadirachtin of 10-19% purity; (g) redissolving the product obtained in step (f) in a solvent and adding the solution to petroleum ether or hexane at ambient temperature gradually under stirring yielding a white solid which after filtration and drying under vacuum at 65°C. resulting in azadirachtin having 15-26% purity as a white powder (h), dissolving the azadirachtin (10-19%) from step (e) in an organic solvent and subjecting to column chromatography (silica gel) by stepwise elution using different compositions of hexane/petroleum ether and ethyl acetate leading to solid azadirachtin powder up to 49% (i) dissolving the azadirachtin having up to 49% purity in methanol. ethanol or acetonitrile and subjecting it to HPLC (C18 column) to produce azadirachtin of purity up to 88% in a solid powder form, and (j) and stirring the product of step (i) with solvents and emulsifiers with or without synergist and UV stabiliser to obtain the emulsifiable concentrate.

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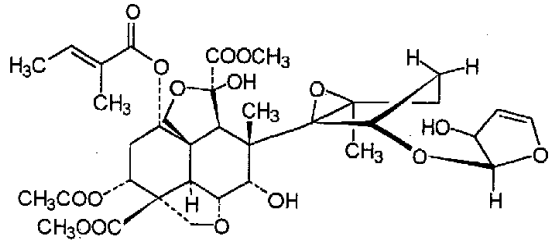
Invention Title: Pesticidal Dry Powder Formulation

The following statement is a full description of this invention, including the best method of performing it known to me/us:-

Pesticidal Dry Powder Formulation

The present invention relates to an azadirachtin preparation in the form of dry solid powder having purity up to 88%, an emulsifiable concentrate comprising up to 30% by weight of azadirachtin and a process for producing such dry solid azadirachtin preparation and emulsifiable concentrate directly from neem seeds/kernels. The dry solid azadirachtin powder prepared by the process of this invention is suitable for insect pest control formulations for use in Agriculture, Veterinary and Public Health from neem (*Azadirachta indica* A. Juss) seeds/kernels. The concentrate prepared by the process of the invention is also used directly as insect pest control agent.

Azadirachtin has structure:



Azadirachtin has been termed as "Azadirachtin A" subsequently, since a number of related compounds designated "Azadirachtins (B-I)" and "Azadirachtin K" have been isolated by different groups (Rembold H, in Economic and Medicinal Plant Research; Eds., Govindachari, TR Sandhya, G and Ganeshraj, SP, Indian Journal of Chemistry, 31A, 295, 1990; Wagner H and Norman, R, Academic Press, New York, 3, 7, 1990; Govindachan TR, Sandhya, G, Ganeshraj, SP, Journal of Natural Products, 55, 596, 1992).

There are several broad spectrum highly toxic organic synthetic insecticides currently in use, for the control of insect pests of food and commercially important crops. Besides, effectively controlling the target pests, they also destroy the natural enemies of pests and other beneficial insects. Insects also develop resistance to some of them owing to indiscrete use. There are also instances of toxic effects of residues of some of the synthetic insecticides for the consumers of the product due to poor biodegradability. Therefore, there is a need for environmentally compatible insecticides possessing activity at low concentrations and selective toxicity to insect, pests, low toxicity to plants and mammals, desired stability and economic viability.

Neem trees are widely distributed in India and some regions of Asia, Africa and Australia. Neem leaves, neem seeds, neem oil and neem cake are traditionally used in India from a long time for insect pest control. Of these,

neem seeds constituting an annually renewable natural source, are associated with highest insect pest control properties.

There are numerous examples reporting the insect antifeedant and insect growth inhibitory properties of azadirachtin for a variety of insect pests (For examples, Butterworth, JH and Morgan, ED, J. Chem. Soc. Chem. Communs. 23, 1968; Leuschner, K, Naturwissenschaften, 59, 217, 1972; Ruscoe, CNE, Nature, Lond., 236, 466, 1972; Schmutterer, H and Rembold, H, Z. Angew. Ent. 2, 179-188, 1980; Warthen, J. Jr., ARMNE-4 USDA, SEA, Agricultural Reviews and Manuals, 1979; Kubo, I and Klocke, JA, Agricultural and Biological Chemistry, 46, 1951, 1982; Champagne, DE, Koul, O, Isman, MB, Scudder, GE and Towers, GHN, Phytochemistry, 31, 377, 1992).

Azadirachtin has also been reported to be non mutagenic (Jacobson, M, Proceedings of the First International Neem Conference, Rottach Egern, 33, 1980; in Natural pesticides from the neem tree, *Azadirachta indica* A. Juss, Schmutterer, H, Ascher, KRS., Eds., German Agency for Technical Cooperation, Eschborn, Germany, 1981) and it appears to have no apparent mammalian toxicity (Nakanishi, K, Recent Advances in Phytochemistry, 5, 283, 1975; Morgan, ED, Proceedings of the First International Neem Conference, Rottach-Egern, 43, 1980; in Natural pesticides from neem tree, *Azadirachta indica* A. Juss, Schmutterer, H, Ascher, KRS, Rembold, H, Eds., German Agency for Technical Cooperation, Eschborn, Germany, 1981).

As a result of the above studies, azadirachtin has been considered to be a promising environmentally compatible insect pest control against for plant protection. It has not come to commercial use because it is expensive to isolate it in a pure form from the neem seed/kernel extract and it is a very complex molecule for an economical chemical synthesis. Azadirachtin has also been found to degrade rapidly due to environmental factors such as W radiation in sunlight, heat, air, moisture, acidity and enzymes present in foliar surfaces (Sundaram, KMS and Curry, J, Journal of Pesticide Science, 41, 129, 1994).

Several groups have investigated the insect antifeedant and insect growth inhibitory properties of solvent extracts of neem seeds or kernels against various insect pests in the laboratory and for the protection of a number of crops against their insect pests under field conditions as they are relatively less expensive and more stable in comparison with pure azadirachtin, leading to promising results (For Examples, Schmutterer, H, Ascher, KRS, Rembold, H, Eds., Natural pesticides from the neem tree, *Azadirachta indica* A. Juss, German Agency for Technical Cooperation, Eschborn, Germany, 1981, 297 pp. Proceedings of the 1st International Neem Conference, Rottach-Egern, 1980; Schmutterer, H, Ascher, KRS, Eds., Natural Pesticides from the neem

tree, *Azadirachta indica* A. Juss and other tropical plants, 583 pp. German Agency for Technical Cooperation, Eschborn, Germany, 1984; Proceedings of the 2nd International Neem Conference, Rauischholzhausen, 1983; Schmutterer, H, Ascher, KRS, Eds., Natural pesticides from neem tree, *Azadirachta indica* A. Juss, and other tropical plants, Germany agency for Technical Cooperation, Eschborn, Germany, 703 pp. 1987; Proceedings of Third International Neem Conference, Nairobi, 1986).

However, solvent extracts of neem seeds or kernels are rather complex mixtures of several compounds requiring standardisation with respect to azadirachtin for reproducible biological activity and performance against insect pests under field conditions. In the prior art, a number of solvents and different temperatures have been explored for extraction of azadirachtin from neem seeds/kernels. Some of these processes are referred below for information.

In the prior art, Butterworth and Morgan (Butterworth, JH and Morgan, ED, J. Insect Physiol., 17, 969, 1971) prepared azadirachtin from neem seeds (2kg). involving (1), extraction with ethanol (170g); (2), partition of the concentrate of ethanol extract between methanol and light petroleum; (3), chromatography (Floridin earth) of partition product (70g.) from methanol phase leading to azadirachtin containing fractions (2g); (4), preparative layer chromatography (PLC) of azadirachtin containing fractions from step 3 resulting in azadirachtin (1.5g). The product obtained from step 2 in this process (76g) would be economical to obtain but it would be viscous and oily due to the presence of water soluble compounds and therefore, it is not suitable for preparing good formulations;. requiring further processing by less expensive techniques for the preparation of a dry powder enriched in azadirachtin.

In the prior art, Uebel, Warthen, Jr. and Jacobson (Uebel, EC, Warthen, Jr. JD and Jacobson, M, J. Liq. Chromatogr., 2, 878, 1979) have isolated azadirachin (2kg batches, 90% purity, 8.7g) from neem seeds/kernels (48.2kg) involving the following steps (1), grinding neem seeds/kernels (2kg batches) in hexane in a Waring blender, filtration of the homogenate to give a residue (marc); (2), soxhlet extraction of powdered marc (1.1kg batches) with acetone (24h); (3), washing of acetone extract with (a) hexane, (b), water and (c), hexane; (4), treatment of washed acetone extract with 70/30, 75/25, methanol/water; (5), treatment of 70/30, 75/25 methanol/water soluble parts with 75/25, diethyl ether/acetone to give 75/25, diethyl ether/acetone soluble azadirachtin containing fractions (102.8g); (7), chromatography (Phase-bonded C-18, Hi-florisil) of diethyl ether/acetone fractions leading to azadirachtin (8.7g 90% purity). The product obtained after treatment of acetone extract with

70/30, 75/25, methanol/H_2O at step 5 by this process requires modification, avoiding diethyl ether a highly inflammable solvent and difficult for solvent recovery and repetitive use in large scale operations. Furthermore, the process details require greater simplification in steps (1), (2) and (3) for large scale operations such as, pre-extraction of neem seed kernels with hexane, before extraction with acetone, should also be avoided since azadirachtin is unstable at the boiling point of acetone (57°C) compared to ambient temperature. Acetone is a low boiling solvent and it is also not an excellent solvent for recovery and repetitive use in large scale operations economically. This method is therefore, not well suited for producing enriched dry azadirachtin powder because the operations are too many, hazardous, uneconomical and inconvenient for large scale preparations.

In the prior art, Feuerhake (Feuerhake, KJ, Proceedings of Second International Neem Conference, Rauischholozhausen, 103, 1983; in Natural Pesticides from the neem tree and other Tropical plants, Schumutterer, H and Ascher, KRS, Eds., German Agency for Technical Cooperation, Eschborn, Germany, 1984), investigated the suitability of the technical solvents (a), methyltertiarybutylether (MTB); (b), methylisobutylketone (MIBK); (c), methylethylketone (MEK), (d), water; (e), methanol; (f), azeotropic mixture of methanol and MTB, (AZT); (g), acetone and (h), butanol and they have recommended AZT for the preparation of azadirachin enriched extracts and found that water is not a convenient solvent for extracting azadirachin since its solubility in water is low. Use of neem oil and p-aminobenzoic acid for protection of azadirachtin is also a prior art. Some examples of other commercial formulations based on neem seeds/kernels/oil are Azatin, Neemguard which is a neem oil formulation, Neemgold containing 300ppm azadirachtin and Neemazal F.

The procedure consists of the following steps; (1), ground neem seed kernels were first extracted in a soxhlet with petroleum ether to remove fatty matter; (2), the extraction was continued with solvents such as MIBK and MTB or acetone or MeOH or AZT or MEK or butanol for 10h; (3), the residue from AZT extract after removal of the solvent was treated with methanol; (4), the methanol soluble portion from step 3, was subjected to liquid-liquid extraction with methanol 50% and light petroleum giving rise to AZT-VR-NR in a yield of 1-1.5% which is expected to be enriched in azadirachtin.

It is desirable to avoid pre-extraction of neem seed kernels with petroleum ether and avoid extraction in a soxhlet with polar solvents at high temperature, since, azadirachtin is not quite stable at these temperatures. In this process, the physical state and stability of AZT-VR-NR were not defined It

will be the starting point for the preparation of a dry solid-enriched in azadirachtin suitable for formulations.

US 4 556 562, involves extraction of neem seed kernel powder with ethanol at 60-90°C. to give ethanol extract containing 5000-10 000ppm of azadirachtin which was treated with nonionic emulsifier and diluted to 2000-4000ppm of azadirachtin in the pH range 3.5-6 and p-aminobenzoic acid and neem oil as stabilisers. Extraction of neem seeds with ethanol has been carried out earlier by Butterworth and Morgan for the isolation of azadirachtin (Butterworth, JH and Morgan, ED, J. Insect Physiol., 17, 969, 1971). Sankaram and coworkers have obtained a fraction enriched in azadirachtin from ethanol extract of defatted neem seeds (soxhlet), dissolved it in acetone, treated it with emulsifier Teepol and diluted it to 0.1 % with water and the resulting formulation protected sorghum and pearl millet crops against their insect pests (Sharma, HC, Leuschner, K, Sankaram, AVB, Gunasekhar D, Marthandamurthi, M, Bhaskariah, K, Subrahmanyam, M and Sulthana, N Proc. 2nd Int. Neem Conf. Rauischholzhausen, 291, 1983; in Natural Pesticides from the neem tree, Eds., Schmutterer, H, Ascher, KRS, German Agency for Technical Cooperation, Eschborn, Germany, 1984).

Yamasaki, Klocke, Stone and Darlington (Yamasaki, RB, Klocke, JA, Stone, GA and Darlington MV, J. Chromatogr. 18, 467, 1986) have isolated azadirachtin from neem seeds (1kg) to obtain azadirachtin (56mg, 99% purity) involving the following steps; (1), neem seeds suspended in hexane (2L) were stirred occasionally at ambient temperature for several hours and the hexane extract was decanted and the process was repeated three more times; (2), the defatted marc from step 1 was extracted six times with methanol (2L) successively, in the same manner as with n-hexane; (3), the filtrate of combined methanol extracts was concentrated *in vacuo* to give an orange tar (78g); (4), the orange tar from step 4 was redissolved in methanol (2L) and diluted with distilled water (2L) under stirring; (5), the aqueous methanol mixture from step 4 is extracted three times with equal portions of n-hexane followed by/three equal quantities of dichloromethane; (6), the combined dichloromethane extract is subjected to flash chromatography (Silica gel, eluent, diethyl ether/methanol) to give amorphous azadirachtin (7.3%, 7.4g), as an orange solid. However, its stability and its suitability for pesticidal applications have not been explored; azadirachtin (7.3%) has been enriched further to azadirachin (26%, yield; 1.26g) by flash chromatography (ODS column, mobile phase, methanol/H_2O, 3/2). The physical state and stability of azadirachtin (26%) have also not been explored; (8), azadirachin (26%) from step 7 is subjected to preparative HPLC to give azadirachin (70%) (silica gel, isopropanol/n-hexane 1/3, yield: 0.280g); (8), azadirachin (70%) was

subjected to phenyl preparative HPLC to give azadirachtin (99%, mobile phase, acetonitrile/H_2O, 3/7, yield; 56mg). Although, this procedure can be used for preparation of azadirachtin (5-30%) suited for pesticidal applications it involved (1), stirring occasionally, ground neem seeds in hexane for several hours and repeating again three times with hexane, six times with methanol decanting every time, successively and this procedure is not convenient and economical for large scale operations and it requires drastic revision; (2), defatting of neem seeds in step 2 with hexane has to be eliminated if possible, since it can reduce the extraction time and cost of production of azadirachtin.

In this process, azadirachtin (7.3%) orange solid, yield 0.74%) obtained by flash chromatography (silica gel) of dichloromethane extract in step 4 is a candidate for novel formulations; however, flash chromatography is an expensive procedure and the yield is low. It is desirable to optimise the purification of dichloromethane extract obtained after step 5 avoiding chromatography leading to a dry solid powder enriched in azadirachtin in a superior yield. It is also desirable to avoid a low boiling environmentally incompatible solvent like dichloromethane. Solvent recovery is poor for large scale operations. Substantially, this procedure is not suited for economic production of solid azadirachtin powder suitable for pesticidal formulations.

Schroeder and Nakanishi (Schroeder, DR and Nakanishi, K, J. Nat. Prod., 50 242, 1987) have described isolation of azadirachtin from neem seed kernels involving the following steps; (1), neem seed kernels (2.0kg) were ground with hexane (2L) in a commercial Waring blender (10min) to a fine powder and allowed to settle for 1h, agitated again and filtered under vacuum to give a neem seed kernel extract residue; (2), the procedure described in step 1 is repeated four times successively resulting in a defatted neem seed kernel cake which is extracted with 95% ethanol (2L), five times by the procedure described in step 1 by percolation within the blender for 8-12h; (3), the combined ethanol extracts after concentration in vacuum yielded a dark viscous residue (185g); (4), the ethanol extract residue from step 3, was partitioned between hexane and methanol/H_2O, 95/5; (5), the 95/5 methanol/water phase (138g) was partitioned with water and ethyl acetate successively; (6), the ethyl acetate phase (59g) was subjected to silica gel filtration with ethyl acetate to give a product (52g) enriched in azadirachtin; (7), the product (52g) from step 6 was subjected to vacuum liquid chromatography (EtOAc/hexane, 3/1) yielding azadirachtin fractions (13g); (8) crystallisation of azadirachtin fractions from carbon-tetrachloride gave crude azadirachtin (8.5g); (9), flash chromatography of the product from step 8 ($CHCl_3$/CH_3CN, 3/1) gave azadirachtin (5g). Extraction by grinding of neem seed kernels with hexane and ethanol in a Waring blender as described in this

procedure is hazardous, uneconomical, and inconvenient for the large scale preparation of azadirachtin requiring modifications. In this procedure, the residue from ethyl acetate extract in step 6 (52g) is the starting point for preparation of dry solid enriched in azadirachtin for novel. formulations without losing the yield preferably, by economic solvent fractionation techniques. Defatting with hexane before extraction with 95% ethanol can be avoided. The steps silica gel filtration and vacuum liquid chromatography to get azadirachtin enriched fractions are difficult to optimise in large scale for preparation of purer azadirachtin required for novel pesticide formulations and it requires modification. The procedure is not suitable for large scale operations.

EP 0311 284, A2 (1988) reports a procedure for the preparation of azadirachtin (25% yield; 0.25%) as a pale yellow residue for the preparation of hydrogenated azadirachtin as an insecticide. This procedure has too many time consuming and uneconomic operations and the yield of the desired product is low. It has to be simplified and optimised to convert it into a manufacturing process for dry solid enriched in azadirachtin suitable for novel formulations.

Govindachari, Sandhya and Ganeshraj (Govindachari, TR, Sandhya, G and Ganeshraj, SP, Chromatographia, 31, 303, 1991) have prepared azadirachtin A involving the following steps; (1), defatting of powdered neem seed kernels (1kg) with hexane (3L); (2), extraction of defatted powdered neem kernels with refluxing 95% ethanol (a), (1L) and (b), (0.5L), (3), partition of ethanol concentrate (95g) obtained after removal of solvent from combined ethanol extract dissolved in 90% methanol with petroleum ether (100mL) thrice; (4), treatment of residue after removal of solvent from methanol extract from step 3, with ethyl acetate; (5), washing of ethyl acetate extract with water to remove proteins, carbohydrates; (6), removal of solvent from ethyl acetate extract to give azadirachtin (25%, 24.5g); (7), preparative HPLC of azadirachtin (25%, 4g) on RP 18 column to give azadirachtin A+D (340mg); (8), preparative HPLC of azadirachtin A+D to give azadirachtin A (160mg).

In this procedure, the physical state of the product containing 25% azadirachtin is not defined. Furthermore, the defatting step has to be eliminated. The extraction methodology requires modification for manufacturing dry azadirachtin powder suitable for novel formulations, since azadirachtin is known to be unstable at high temperatures, extraction at refluxing temperature of ethanol has to be avoided. Refluxing of powdered neem kernels with 95% ethanol is not convenient for large scale extractions, economically.

Kleeberg, reported the preparation of a stable azadirachtin rich powder from neem seeds (Kleeberg, H, DE 4 109 473, 1992, Chemical Abstracts, 118, 18002, s, 1993) involving (1) extraction of neem seeds with water; (2), extraction of aqueous extract with ethyl acetate; (3), treatment of ethyl acetate concentrate with petroleum ether resulting in enriched azadirachtin powder. This process is not suitable for preparing powder enriched in azadirachtin economically, due to the fact that water is not a suitable solvent for extraction of azadirachtin from neem seeds economically, since solubility of azadirachtin in water is very poor compared to solvents such as methanol and ethanol which extract azadirachtin from neem seeds/kernels efficiently. The yield of the azadirachtin powder will be low by this procedure.

Majority of the above processes employ initial extraction of seeds or kernels either with petroleum ether/hexane/heptane. This is followed by extraction with polar solvents such as acetone or methanol or ethanol at ambient temperature or at refluxing temperature of the solvent or in a soxhlet extraction apparatus. Furthermore, these methods aim at extraction of expensive pure azadirachtin required for determination of its structure, chemistry, biological activity and analytical studies using sophisticated and elaborate, Preparative Layer Chromatography (PLC), Column Chromatography and High Performance Liquid Chromatography (HPLC) techniques, irrespective of the applicability of the intermediate stages of the extracts for their azadirachtin content. physical state. suitability for storage, stability, and for preparation of their formulations for practical insect pest control. In fact, in the above said processes, neem seed and kernel extracts fairly rich in azadirachtin content have been obtained but their physical state, suitability for storage, stability, formulation and economic production have not been explored and they are not well suited for large scale preparation economically. For storage, stability and formulation, it is desirable to have a free flowing powder containing 5-30% of azadirachtin, and for the preparation of formulations such as Emulsifiable concentrates (EC), Wettable dispersable powders (WDP), dusts, granules, aerosols, controlled release formulations etc., the extracts should be preferably devoid of even traces of water soluble compounds such as inorganic salts, carbohydrates, proteins and colouring matters as these would present problems for example, in designing a suitable solvent for dissolution of the active ingredient for the preparation of an emulsifiable concentrate and stability for practical applications.

Viewed in this context, the concentrates of crude polar solvent extracts of neem seeds or kernels obtained after the removal of solvents by the above process are usually oily and gummy due to the presence of fatty constituents and water soluble compounds such as tannins, organic carboxylic acids,

carbohydrates, proteins, organic and inorganic salts, pigments etc., and they do not possess good keeping qualities. These concentrates also present problems of solubility in the preparation of Emulsifiable concentrate (EC) formulations and caking in the preparation of Wettable dispersable powders (WDP) formulations, dusts, and granular formulations.

In spite of the above advances, there is a need for a procedure for the preparation of a neem seed/kernel extract in a dry powder form in a large scale conveniently with 5-30% of azadirachtin, stability, good solubility in organic solvents suitable for EC formulations and compatibility with carrier materials for producing WDP formulations useful for insect pest control.

Acetone solutions of azadirachtin when exposed to sunlight for seven days and sixteen days gave >50% and complete reduction respectively, in insect antifeedant activity against 1st instar fall army worm (*Spodoptera frugiperda*) implicating the destruction of azadirachtin (Stokes, JB, Redfern, RE, J. Env. Sci. Health, Part A, 1982, A, (17), 57-65; Chemical Abstracts, 96: 137955). Photodegradation of azadirachtin in sunlight was arrested to <25% for two weeks by Neem, Angelic, Castor and Calanus oils.

Extraction of neem seeds with ethanol, isolation of azadirachtin from the ethanol extract, and insect antifeedant properties of azadirachtin for desert locust have been reported much earlier by Butterworth and Morgan in 1971 (Butterworth, JH and Morgan, ED, J. Insect Physiol. 17, 969, 1971).

Neem extracts enriched in active compounds such as azadirachtin, salannin, desacetyl nimbin and nimbin have also been formulated using surface active agents and special solvents and these were found to be effective agents and special solvents and these were found to be effective in Field and Laboratory tests (Feuerhake, K and Schmutterer, H, Z. Pflanzen Krank Pflanzen Schutz. 1985, 92(6) 643-9, Chemical Abstracts, 104, 163705h, 1986) as insecticides.

A mixture of citronella oil, neem seed extract containing 5% of azadirachtin, triclosan, DEET, di-pr-isocinchomerate, traces of lemon grass oil and ethanol was heated at 54°C. for 14 days resulting in an insect repellent. During heating, azadirachtin disappeared and new compounds were formed (Henry, GV, WO91/5970 Chemical Abstracts, 118; p. 75393 h).

The azadirachtin content of an aromatic petroleum distillate solution containing 3.2% of azadirachtin and 10% of 1,2-epoxyoctane, was reduced to 2.8% on storage for twenty eight days (Butler, BJ, Ellenberger, WP, Omilinsky, BA WO94/02019; Chemical Abstracts, 120, p.263865 q.

Azadirachtin has also been found to degrade rapidly due to environmental factors such as UV radiation in sunlight, heat, air, moisture, acidity and enzymes present in foliar surfaces (Sundaram, KMS and Curry, J, Pesticide

Science, 1944, 41, 129). From these results it is clear that there is a need for stabilised formulations of neem seed/kernel/oil extracts containing azadirachtin for crop protection, stored grain protection and in public health.

We have observed due to our sustained research in this area, that the major quantity of azadirachtin can be extracted directly from powdered neem seeds/kernels with polar solvent such as methanol or aqueous methanol or ethanol (rectified spirit) or aqueous ethanol at ambient temperature wherein the fatty oils are predominantly retained with the seed/kernel residue so that initial extraction of powdered neem seeds/kernels with hexane/petroleum ether (bp 60-80°C) for the removal of fatty oils is avoided. By carrying out the extraction at ambient temperature, thermal degradation of azadirachtin during extraction is minimised.

We have found that, it is possible to disintegrate dry neem seeds/kernels to a suitable powder smoothly in the range of particle size BSS-7 (0.2mm) - BSS-72 (2.4mm) for the large scale solvent extraction of powdered neem seeds/kernels.

Thus, the present invention utilises directly, methanol or aqueous methanol or ethanol or aqueous ethanol for the extraction of azadirachtin from powdered neem seeds/kernels, in a column by continuous percolation. The present invention employs continuous solvent percolation at ambient temperature to avoid decomposition of azadirachtin at higher temperature and it is convenient for modular operations.

The present invention specifically, deals with the manufacture of a neem seed/kernel extract containing up to 88% of azadirachtin as a dry powder directly from neem seeds/kernels involving continuous batch extraction using methanol or aqueous methanol or ethanol or aqueous ethanol, concentration of these extracts, solvent fractionation of the concentrates, fractional precipitation at ambient temperature, column chromatography and HPLC.

The present invention also deals specifically with the preparation of emulsifiable concentrate formulation of dry powder enriched in azadirachtin up to 30% for insect/pest control application.

Accordingly, the present invention provides a process for the preparation of azadirachtin, in a dry solid powder form having a purity up to 88% which is useful directly for pesticide formulations, from neem seeds/kernels, which comprises;

(a) disintegrating the neem seeds/kernels into a powder; (b) subjecting the said powder to continuous batch percolation using methanol or aqueous methanol or ethanol or aqueous ethanol at ambient temperature in a column; (c) concentrating the extract and stirring the concentrate with petroleum ether or hexane and phase separating by conventional methods; (d) stirring the denser phase containing major quantity of azadirachtin with a water immiscible organic solvent and water as required depending on the solvent used for extraction and phase separating by conventional methods; (e) concentrating the organic phase and gradually adding the concentrate to petroleum

ether or hexane under stirring at ambient temperature (f), filtering under suction and drying under vacuum at a temperature in the range of 25-65°C to obtain a neem seed or kernel extract as a powder containing azadirachtin of 10-19% purity; (g) redissolving the product obtained in step (f) in an organic solvent; (h) adding the solution to petroleum ether or hexane under stirring yielding a white powder, which after filtration and drying under vacuum at 65°C resulting in azadirachtin having 15-26% purity as a white powder (i), dissolving the azadirachtin from step (f) in an organic solvent and subjecting to column chromatography by stepwise elution using different compositions of hexane or petroleum ether and ethyl acetate leading to solid azadirachtin powder of at least 49% and (j) finally dissolving the azadirachtin having up to 49% purity in methanol, ethanol or acetonitrile and subjecting it to HPLC to yield azadirachtin of purity of up to 88% as a solid powder.

According to another aspect, the present invention resides in a process for the preparation of emulsifiable concentrate comprising up to 30% by weight of azadirachtin from neem seeds/kernels which comprises stirring the azadirachtin powder having purity up to 88% with solvents or mixtures thereof, emulsifiers or emulsifier continuously with or without synergist and with or without UV stabiliser and thereby obtaining the clear emulsifiable concentrate.

The solvents used for dissolution of powder may be selected from aromax (petroleum distillate containing aromatic compounds), 2-butanone, cyclohexanone, dimethlformamide, dimethylphthalate, dioctylphthalate , isobutanol, isobutylmethylketone, isopropanol, Solvent C-IX (petroleum distillate containing higher aliphatic alkanes isoalkanes, in the range C_5, C_7, C_8, C_9 to C_{74},) and xylene individually or as suitable mixtures. The emulsifier used may be selected from commercially available ionic or nonionic emulsifiers, such as, Cresolox 3409, Emulsol MAL, Emulsol 172 RH, Igesol (CABS), Ethylene oxide condensate, 10mole. Triton X100 and Tween 80. The synergist used is piperonyl butoxide. 2-hydroxy-4-octoyloxy benzophenone is used as a UV stabiliser optionally until a brown homogeneous stable liquid is obtained. The resulting concentrate can be used as a spray fluid for insect pest control after dilution with water.

The following steps are involved in the process of the present invention:

a. Well dried Neem seeds/kernels are disintegrated to a powder of suitable size using a mill.

b. The seed/kernel powder so obtained, from step (a) is packed into a suitable column and subjected to continuous percolation with methanol or ethanol (rectified spirit) or methanol or ethanol containing 20% water at ambient temperature and the most preferred solvent is methanol.

c. The extract in step (b) is concentrated at atmospheric pressure or under vacuum and the preferred condition is under vacuum.

d. The concentrate from step (c) is stirred with an organic solvent such as petroleum ether or hexane and the two phases viz. denser phase and lighter phase are separated.

e. The denser phase obtained from step (d) containing azadirachtin is stirred with water and a water immiscible organic solvents such as benzene, 2-butanone, chloroform, dichloromethane, dichloroethane, diisopropyl ether, ethyl acetate, methyltertiarybutylether and toluene and the phases are separated. The preferred water immiscible organic solvent is ethyl acetate.

When methanol or ethanol containing 20% water is used for extraction of powdered neem seed kernels use of water along with the water immiscible solvent in this step is not required.

f. The organic phase in step (e) is concentrated at atmospheric pressure or under vacuum and preferred condition is under vacuum.

g. The concentrate obtained from step (f) is added gradually to petroleum ether (bp 60-80°C) or hexane under stirring or at reflux temperature and filtered to give a brownish yellow solid which is dried under vacuum at 65°C which contains 10-19% of azadirachtin and it can be formulated for protection of several crops such as cotton, rice, groundnut, brinjal, cabbage, castor, citrus species, coffee, potato, pulses, sorghum, sugarcane, tobacco, etc. and the stored grains against their insect pests.

h. The product from step (g) is dissolved in dichloromethane or ethyl acetate and the solution is added gradually to petroleum ether (bp 60-80°C) or hexane under stirring and the resulting solid was filtered and dried at 65°C in vacuum. The resulting white powder contains 15-26% of azadirachtin. The resultant product is useful for insect pest control formulations. The most preferred solvents used in this step are ethyl acetate and petroleum ether (bp 60-80°C).

i. The product from step (g) is dissolved in dichloromethane/ethyl acetate and subjected to open column chromatography (Silica gel) using mixtures of hexane or petroleum ether (bp 60-80°C) and ethyl acetate as eluents stepwise leading up to 49% azadirachtin after removal of the solvent in vacuum as a white solid

j The product from step (i) is dissolved in methanol, filtered through a short C18 guard column and the filtrate was diluted and subjected to semi preparative HPLC (C18 column) leading to a white solid containing up to 88% of azadirachtin.

The invention also relates to a process for the preparation of Emulsifiable Concentrate comprising up to 30% by weight of azadirachtin from neem seeds/kernels, which comprises stirring the azadirachtin powder having purity up to 88% with solvents or mixtures thereof, emulsifiers or emulsifier combinations with or without synergist and with or without UV stabiliser and

thereby obtaining the clear emulsifiable concentrate. The organic solvents used in the above process are aromax, 2-butanone, cyclohexanone, dimethylformamide, dimethylphthalate, dioctylphthalate, isobutanol, isobutylmethylketone, isopropanol, solvent C-IX and xylene individually or as suitable combinations. The emulsifiers used are the ones which are commercially available ionic and nonionic emulsifiers such as Cresolox 3409, Emulsol MAL, Emulsol 172 RH, Igesol, calcium alkyl benzene sulfonate (CABS), Ethylene oxide condensates (10mole), Triton X 100, and Tween 80. The piperonyl butoxide is used as a synergist and 2-hydroxy-4-octyloxy-benzophenone is used as UV stabiliser.

Therefore, the present invention provides an economical and convenient process for the manufacture of neem seed/kernel extract containing up to 88% azadirachtin from neem seeds/kernels directly by continuous solvent percolation at ambient temperature, by solvent extraction, concentration, solvent partition, fractional precipitation, as a dry powder suitable for formulation of Emulsifiable concentrates, Wettable dispersible powders, dusts etc. for practical insect pest control. The present invention also provides a process for enrichment of azadirachtin powder (10-26%) by stepwise column chromatography (silica gel) to azadirachtin up to 49% powder which is further enriched to azadirachtin (88%) powder by HPLC (C 18 column) for special applications.

We have observed that it is not necessary to extract pulverised neem seeds or kernels first with petroleum ether or hexane followed by extraction with methanol or aqueous methanol or ethanol or aqueous ethanol for the extraction of azadirachtin as the fatty oils are not extracted by these solvents at ambient temperature significantly from the powdered neem seeds and kernels and they are in fact, retained predominantly with the residual seed or kernel powder and they do not interfere with the isolation of azadirachtin in subsequent stages.

In the prior art, the concentrated polar solvent extract yielded oily or gummy solid, or viscous mass even after comparable purification steps with lesser content of azadirachtin. The present process, an extract containing up to 88% of azadirachtin is obtained in the form of powder which can be easily used for preparing insect pest control formulations.

Extraction of azadirachtin by continuous solvent percolation directly with a polar solvent in a column packed with suitably disintegrated neem seeds/kernels at ambient temperature by the present economical and conveniently upscalable process is an improvement over the processes of prior art, which utilise hazardous, uneconomic and not conveniently upscalable, repetitive operations such as, disintegration and extraction of neem

seeds/kernels suspended in volatile and hazardous organic solvents using commercial blenders and stirring. Continuous solvent percolation of disintegrated neem seeds/kernels directly, with a polar solvent carried out economically avoiding defatting in the present economic and conveniently upscalable process at ambient temperature retaining majority of lipid constituents interfering with the extraction and enrichment of azadirachtin with the residual neem seed/kernel powder is an improvement over some of the processes of prior art, which compulsorily used avoidable solvent extraction of neem seeds/kernels, with petroleum ether/hexane for defatting prior to extraction with polar solvents for the isolation of azadirachtin.

Extraction of azadirachtin at ambient temperature in the process of the present invention arresting degradation of azadirachtin due to its known thermal instability is an improvement over extractions carried out at higher temperature or in a soxhlet extraction equipment at the boiling point of the solvents used in the prior art processes. Preparation of azadirachtin powder from crude polar solvent extract of neem seeds/kernels containing azadirachtin in the process of present invention avoids expensive and unnecessary repetitive operations for the preparation of similar extracts of prior art. Preparation of azadirachtin (15-26%) powder suitable for formulations by gradual addition of concentrate of ethyl acetate extract to petroleum ether (bp 60-80°C)/hexane under stirring followed by filtration and drying of precipitated azadirachtin powder in two successive operations by the present economical and conveniently upscalable process is an improvement over the processes of prior art which employed expensive, time consuming and some avoidable solvent extraction and partition operations, chromatographic techniques and hazardous solvents such as diethyl ether for the preparation of similar products.

The present process is an improvement over a procedure described in DE 109 473 (1992) for the preparation of stable azadirachtin rich insecticidal powder from neem seeds (Kleeberg, H., Chemical Abstracts? 118, 18, 0002, s) involving (1), extraction of neem seeds with water; (2), extraction of aqueous extract with ethyl acetate; (3), precipitation of azadirachtin powder from the ethyl acetate concentrate by addition of petroleum ether, since the yields of the final product are expected to be low in view of poor solubility of azadirachtin in water.

Enrichment of azadirachtin (10-26%) powder to azadirachtin up to 49% powder by the present process involving stepwise elution with different compositions of petroleum ether and ethyl acetate by column chromatography is an improvement over the prior art which utilised hazardous solvents such as

diethyl ether or repeated employment of expensive chromatographic techniques.

Enrichment of azadirachtin up to 49% powder to azadirachtin (88%) according to the process of the present invention by a single easily repeatable HPLC step (C 18 column) (due to the removal of undesirable polar constituents deteriorating the column in earlier steps) is an improvement over the prior art, which involves laborious PLC, which is not upscalable, time consuming solvent partition operations and hazardous solvents such as diethyl ether, and number of expensive chromatographic experiments.

According to the process of the present invention azadirachtin having different purities in a dry powder form which is suitable for pesticidal formulation is obtained. Further, the dry powder so obtained is stirred with organic solvents or their derivatives, emulsifiers or their combinations, UV stabiliser and synergist (optionally).

The invention is described with reference to the examples given below which are given to illustrate the invention and therefore should not be considered to limit the scope of the invention.

Example 1

Well cleaned dry neem seeds were pulverised in a multi mill to a coarse powder having particle size ranging from BSS-7 (0.2mm.) to BSS-72 (2.4mm). The resulting neem seed powder (200g)was packed into a glass column and extracted continuously by percolation of methanol at ambient temperature. The methanol extract (1000mL) was concentrated at atmospheric pressure. The concentrate (40mL) was stirred with an equal volume of petroleum ether (bp 60-80°C) in a stirred vessel and allowed to settle. The two phases were separated in a phase separator. The denser phase was stirred with ethyl acetate (40mL) and water (20mL) in a stirred vessel, allowed to settle and the lighter ethyl acetate phase was separated. The denser phase was again stirred with ethyl acetate (20mL) and processed in the same manner to give the lighter phase. Both the lighter phases were combined and concentrated at atmospheric pressure. The concentrate so obtained was gradually added to petroleum ether (b.p 60-80°C) (40mL) under stirring at room temperature or under refluxing conditions and the solid was filtered under suction and dried in vacuum (65°C) leading to brownish yellow solid (3 65g) containing 10.84% of azadirachtin by HPLC analysis.

The product containing 10.84% of azadirachtin (8g) was dissolved in ethyl acetate and the solution was applied to a column packed with silica gel (240g less than 0.08mm), slurry made with a composition of petroleum ether (bp 60-80°C)/ethyl acetate (9/1). The column was eluted successively with

different compositions of petroleum ether (bp 60-80°C)/ethyl acetate starting from 9/1, 8/2, 7/3, 6/4, 5/5, 4/6, 3/7, 2/8, 1/9 and ethyl acetate stepwise and fractions containing 600mL each were collected. Fraction 8 eluting with petroleum ether/ethyl acetate composition (2/8) yielded a white residue (1.5g) after removal of the solvent under vacuum and it contained 30.42% of azadirachtin by HPLC.

The solid containing 30.42% of azadirachtin (500mg), was dissolved in methanol (5mL) and filtered through a short column (Adsorbsil, LC, C18, 100/200 mesh) and the filtrate was made up to 10mL. It was processed by semi-preparative HPLC (μ bondapak, C18 column (ID) X 150mm. using mobile phase - MeOH/H_2O. 6/4 O D range- 2 AUF: Detector- UV 217nm; flow rate - 4mL/min.; sample volume 300μL; attenuation - 1). The eluant of the peak having retention time 22.6min. was concentrated under vacuum and residue was processed leading to a white solid which was dried under vacuum at 65°C and its azadirachtin content was 88%.

The product (1g) containing 10.84% of azadirachtin was enriched to a product containing 17% of azadirachtin by dissolving in ethyl acetate and adding the solution gradually to petroleum ether (bp 60-80°C) under stirring and the resulting solid was filtered and dried at 65°C.

Example 2

Well cleaned dry neem seeds were pulverised in a multi mill to a coarse powder having particle size ranging from BSS-7 (0.2mm) to BSS-72 (2.4mm). The resulting neem seed powder (200g) was packed into a glass column and extracted continuously by percolation of ethanol (rectified spirit) at ambient temperature. The ethanol extract (1000mL) was concentrated at atmospheric pressure. The concentrate (40mL) was stirred with an equal volume of petroleum ether (bp 60-80°C) in a stirred vessel and allowed to settle. The two phases were separated in a phase separator. The denser phase was stirred with ethyl acetate (40mL) and water (20mL) in a stirred vessel, allowed to settle and the lighter ethyl acetate phase was separated. The denser phase was again stirred with ethyl acetate (20mL.) and processed in the same manner to give the lighter phase. Both the lighter phases were combined and concentrated at atmospheric pressure. The concentrate so obtained was gradually added to petroleum ether (bp 60-80°C.) under stirring at room temperature and the solid was filtered under suction and dried in vacuum (65°C.) leading to brownish yellow solid (30g.) containing 9.15% of azadirachtin by HPLC analysis.

The product containing 9.15% of azadirachtin was dissolved in ethyl acetate and the solution was applied to a volume packed with silica gel (240g,

less than 0.08mm) slurry made with a composition of petroleum ether (bp 60-80°C/ethyl acetate (9/1). The column was eluted successively with different compositions of petroleum ether (bp 60-80°C)/ethyl acetate starting from 9/1, 812, 7/3, 6/4, 5/5, 4/6, 3/7, 2/8, 1/9 stepwise and fractions containing 600mL each were collected.

Fraction 8 eluting with petroleum ether (bp 60-80°C)ethyl acetate composition 2/8 yielded a white residue (1.5g) after removal of the solvent under vacuum and it contained 39.7% of azadirachtin by HPLC analysis.

The solid containing 39.7% of azadirachtin (500mg), was dissolved in methanol (5mL) and filtered through a short column (Adsorbsil, LC, C18, 100/200 mesh) and the filtrate was made up to 10mL. It was processed by semi-preparative HPLC (μ bondapak, C 18 column 19mm. (ID) X 150mm. using mobile phase-MeOH/H_2O,. 6/4 O D range- 2 AUF: Detector- UV 217nm; flow rate - 4mL/min.; sample volume - 300μL; attenuation - 1). The eluant of the peak having retention time 22.6min was concentrated under vacuum and residue was processed leading to a white solid which was dried under vacuum at 65°C and its azadirachtin content was 88%.

The product (1g) containing 9.15% of azadirachtin was enriched to a product containing 11.8% of azadirachtin by dissolving in ethyl acetate and adding the solution gradually to petroleum ether (bp 60-80°C) under stirring and the resulting solid was filtered and dried at 65°C.

Example 3

Well cleaned dry neem seeds were pulverised in a multi mill to a coarse powder having particle size ranging from BSS-7 (0 2mm) to BSS-72 (2.4mm). The resulting neem seed powder (200g) was packed into a glass column and extracted continuously by percolation of methanol extract (1000mL) was concentrated at atmospheric pressure. The concentrate (40mL) was stirred with an equal volume of petroleum ether (bp 60-80°C) in a stirred vessel and allowed to settle. The two phases were separated in a phase separator. The denser phase was stirred with ethyl acetate (40mL) in a stirred vessel, allowed to settle and the lighter ethyl acetate phase was separated. The denser phase was again stirred with ethyl acetate (20mL) and processed in the same manner to give the lighter phase. Both the lighter phases were combined and concentrated at atmospheric pressure. The concentrate so obtained was gradually added to petroleum ether (bp 6080°C) under stirring at room temperature and the solid was filtered under suction and dried in vacuum (65°C) leading to brownish yellow solid (3.9g) containing 11.9% of azadirachtin by HPLC analysis.

The product containing 11.9% of azadirachtin was dissolved in ethyl acetate and the solution was applied to a column packed with silica gel (240g, less than 0.08mm) slurry made with a composition of petroleum ether (bp 60-80°C/ethyl acetate (9/1). The column was eluted successively with different compositions of petroleum ether (bp 60-80°C)/ethyl acetate starting from 9/1, 8/2, 7/3, 6/4, 5/5, 4/6, 3/7, 2/8, 1/9 stepwise and fractions containing 60 mL each were collected. Fraction 8 eluting with petroleum ether (bp 60-80°C)ethyl acetate composition 2/8 yielded a white residue (1.5g) after removal of the solvent under vacuum and it contained 31.19% of azadirachtin by HPLC analysis.

The solid containing 31.19% of azadirachtin (500mg), was dissolved in methanol (5mL) and filtered through a short column (Adsorbsil LC, C18, 100/200 mesh) and the filtrate was made up to 10mL. It was processed by semi-preparative HPLC (μ bondapak, C18 column 19mm. (ID) X 150mm. using mobile phase-MeOH/H_2O,. 6/4; O D range 2 AUF: Detector- UV 217nm; flow rate - 4mL/min.; sample volume - 300μL; attenuation - 1). The eluant of the peak having retention time 22.6min was concentrated under vacuum and residue was processed leading to a white solid which was dried under vacuum at 65°C and its azadirachtin content was 88%. The product (1g) containing 11.9% of azadirachtin was enriched to a product containing 15.72% of azadirachtin by dissolving in ethyl acetate and adding the solution gradually to petroleum ether (bp 60-80°C) under stirring and the resulting solid was filtered and dried at 65°C.

Example 4

Well cleaned dry neem seed/kernels were pulverised in a grinder to a coarse powder having particle size ranging from BSS-7 (0.2mm) to BSS-72 (2.4mm). The resulting neem seed and kernel powder (200g) was packed into a glass column and extracted continuously by percolation of methanol at ambient temperature. The methanol extract (1000mL) was concentrated at atmospheric pressure. The concentrate (40mL) was stirred with an equal volume of petroleum ether (bp 60-80°C) in a stirred vessel and allowed to settle. The two phases were separated in a phase separator. The denser phase was stirred with ethyl acetate (40mL) and water (20mL) in a stirred vessel, allowed to settle and the lighter ethyl acetate phase was separated. The denser phase was again stirred with ethyl acetate (20mL) and processed in the same manner to give the lighter phase. Both the lighter phases were combined and concentrated at atmospheric pressure. The concentrate so obtained was gradually added to petroleum ether (bp 60-80°C 40mL) under stirring at room temperature and the solid was filtered under suction and dried in vacuum

(65°C) leading to brownish yellow solid (4.0g) containing 16.16% of azadirachtin by HPLC analysis. The product containing 16.16% of azadirachtin (8g) was dissolved in ethyl acetate and the solution was applied to a column packed with silica gel (200g, Acme less than 0.08mm) slurry made with a composition of petroleum ether (bp 60-80°C/ethyl acetate (9/1). The column was eluted successively with different compositions of petroleum ether (bp 60-80°C)/ethyl acetate starting from 9/1, 8/2, 7/3, 6/4, 5/5, 4/6, 3/7, 2/8, 1/9 stepwise and fractions containing 600mL each were collected. Fraction 8 eluting with petroleum ether (bp 60-80°C)ethyl acetate composition 2/8 yielded a white residue (1.5g) after removal of the solvent under vacuum and it contained 49.24% of azadirachtin by HPLC analysis.

The solid containing 49.24% of azadirachtin (500mg), was dissolved in methanol (5mL) and filtered through a short column (Adsorbsil, LC, C18, 100/200 mesh) and the filtrate was made up to 10mL. It was processed by semi-preparative HPLC (μ bondapak, C 18 column 19mm. (ID) X 150mm using mobile phase-MeOH/H_2O,. 6/4; O D range- 2 AUF: Detector- UV 217nm; flow rate - 4mL/min sample volume - 300μL; attenuation - 1). The eluant of the peak having retention time 22.6min was concentrated under vacuum and residue was processed leading to a white solid which was dried under vacuum at 65°C and its azadirachtin content was 88%.

The product (1g) containing 16.16% of azadirachtin was enriched to a product containing 22.89% of azadirachtin by dissolving in ethyl acetate and adding the solution gradually to petroleum ether (bp 60-80°C) under stirring and the resulting solid was filtered and dried at 65°C.

Example 5

Well cleaned dry neem seed and kernels were pulverised in a grinder to a coarse powder having particle size ranging from BSS-7 (0.2mm) to BSS-72 (2.4mm). The resulting neem seed and kernel powder (200g) was packed into a glass column and extracted continuously by percolation of methanol containing 20% water at ambient temperature. The aqueous methanol extract (1000mL) was concentrated at atmospheric pressure. The concentrate (40mL) was stirred with an equal volume of petroleum ether (bp 60-80°C) in a stirred vessel and allowed to settle. The two phases were separated in a phase separator. The denser phase was stirred with ethyl acetate (40mL) in a stirred vessel, allowed to settle and the lighter ethyl acetate phase was separated. The denser phase was again stirred with ethyl acetate (20mL) and processed in the same manner to give the lighter phase. Both the lighter phases were combined and concentrated at atmospheric pressure. The concentrate so obtained was gradually added to petroleum ether (bp 60-80°C 40mL) under

stirring at room temperature and the solid was filtered under suction and dried in vacuum (65°C) leading to brownish yellow solid (3.75g) containing 19.93% of azadirachtin by HPLC analysis. The product containing 19.93% of azadirachtin (8g) was dissolved in ethyl acetate and the solution was applied to a column packed with silica gel (200g less than 0.08mm) slurry made with a composition of petroleum ether (bp 60-80°C/ethyl acetate (9/1). The column was eluted successively with different compositions of petroleum ether (bp 60-80°C)/ethyl acetate starting from 9/1, 8/2, 7/3, 6/4, 5/5, 4/6, 3/7, 2/8, 1/9 stepwise and fractions containing 600mL each were collected. Fraction 8 eluting with petroleum ether (bp 60-80°C)/ethyl acetate composition 2/8 yielded a white residue (1.5g) after removal of the solvent under vacuum and it contained 45% of azadirachtin by HPLC analysis.

The solid containing 45% of azadirachtin (500mg), was dissolved in methanol (5mL) and filtered through a short column (Adsorbsil, LC, C18, 100/200 mesh) and the filtrate was made up to 10mL. It was processed by semi-preparative HPLC (bondapak, C18 column 19mm (ID) X 150mm using mobile phase-MeOH/H_2O, 6/4 sample range- 2 AUF: Detector- UV 217nm; flow rate - 4mL/min.; sample volume - 300µL; attenuation - 1). The eluant of the peak having retention time 22.6min was concentrated under vacuum and residue was processed leading to a white solid which was dried under vacuum at 65°C and its azadirachtin content was 88%.

The product (1g) containing 19.93% of azadirachtin was enriched to a product containing 26.86% of azadirachtin by dissolving in ethyl acetate and adding the solution gradually to petroleum ether (bp 60-80°C) under stirring and the resulting solid was filtered and dried at 65°C.

Example 6

Well cleaned dry neem seed and kernels were pulverised in a grinder to a coarse powder having particle size ranging from BSS-7 (0.2mm) to BSS-72 (2.4mm). The resulting neem seed powder (200g) was packed into a glass column and extracted continuously by percolation of ethanol at ambient temperature. The ethanol extract (1000mL) was concentrated at atmospheric pressure. The concentrate (40mL) was stirred with an equal volume of petroleum ether (bp 60-80°C) in a stirred vessel and allowed to settle. The two phases were separated in a phase separator. The denser phase was stirred with ethyl acetate (40mL) and water (20mL) in a stirred vessel, allowed to settle and the lighter ethyl acetate phase was separated. The denser phase was again stirred with ethyl acetate (20mL) and processed in the same manner to give the lighter phase. Both the lighter phases were combined and concentrated at atmospheric pressure. The concentrate so obtained was

gradually added to petroleum ether (bp 60-80°C) (40mL) under stirring at room temperature and the solid was filtered under suction and dried in vacuum (65°C) leading to brownish yellow solid (2.8g) containing 16.06% of azadirachtin by HPLC analysis.

The product containing 16.06% of azadirachtin (8g) was dissolved in ethyl acetate and the solution was applied to a column packed with silica gel (240g less than 0.08mm) slurry made with a composition of petroleum ether (bp 60-80°C/ethyl acetate (9/1). The column was eluted successively with different compositions of petroleum ether (bp 60-80°C)/ethyl acetate starting from 9/1, 8/2, 7/3, 6/4, 5/5, 4/6, 3/7, 2/8, 1/9 and ethyl acetate stepwise and fractions containing 600mL each were collected. Fraction 8 eluting with petroleum ether/ethyl acetate composition (2/8) yielded a white residue (1.5g) after removal of the solvent under vacuum and it contained 45.5% of azadirachtin by HPLC analysis.

The solid containing 45.5% of azadirachtin (500mg), was dissolved in methanol (5mL) and filtered through a short column (Adsorbsil, LC, C18, 100/200 mesh) and the filtrate was made up to 10mL. It was processed by semi-preparative HPLC (μ bondapak, C18 column 19mm. (ID) X 150mm. using mobile phase-MeOH/H_2O 6/4; OD range- 2 AUF: Detector- UV 217nm; flow rate - 4mL/min.; sample volume - 300μL; attenuation - 1). The eluant of the peak having retention time 22.6min was concentrated under vacuum and residue was processed leading to a white solid which was dried under vacuum at 65°C and its azadirachtin content was 88%.

The product (1g) containing 16.06% of azadirachtin was enriched to a product containing 20.44% of azadirachtin by dissolving in ethyl acetate and adding the solution gradually to petroleum ether (bp 60-80°C) under stirring and the resulting solid was filtered and dried at 65°C.

Example 7

Dry neem seed extract powder (20g) obtained by any one of the previous examples was slowly added to a well stirred solution of solvent C-IX (51g), cyclohexanone (14g) Triton X 100 (4.12g) Tween 80 (2.94g), Igesol, CABS) (2.94g) and piperonyl butoxide (5g) were taken in a beaker and stirring continued for 1h until a clear brown solution was obtained.

Example 8

Neem seed extract powder (1kg) obtained by any one of examples 1 to 6 was added slowly to a well stirred homogeneous solution of solvent C-IX (2.35kg) cyclohexanone (1kg), Triton x 100 (206.0g) Tween 80 (147g), Igesol CABS (147g) piperonyl butoxide (500g) and 2-hydroxy-4-

octyloxybenzophenone, (25g) taken in a beaker and stirring was continued for 8h until a clear brown solution was obtained.

Example 9

Dry neem seed extract powder (20g) obtained by any one of examples 1 to 6, xylene (51g), 2-butanone (14g) Triton x 100 (4.12g), Tween 80 (2.94g), and Igesol CABS (2.94g), piperonyl butoxide (5g) were taken in a beaker and stirred for 1h until a clear brown solution was obtained.

Example 10

Dry neem seed extract powder (20g) obtained by any one of examples 1 to 6, dimethylphthalate (35g) and cyclohexanone (30g), emulsol MAL (10g) and piperonyl butoxide (5g), were taken in a beaker and stirred until a clear brown solution was obtained.

Example 11

Dry neem seed extract powder (20g) obtained by any one of examples 1 to 6, cyclohexanone (70g) and emulsifier Creslox 3409 (10g) were taken in a beaker and stirred until a clear brown solution was obtained.

Example 12

Neem seed extract power (20g) obtained by any one of examples 1 to 6, cyclohexanone (60g), dioctylphthalate (8g) Emulsol 172 RH (10g), Triton x 100 (2g) were taken in a beaker and stirred until a clear brown solution was obtained.

Example 13

Neem seed extract powder (20g) obtained by any one of examples 1 to 6, isobutyl methyl ketone (56g) dimethylformamide (14g), Cresolox 3409 (10g), were taken in a beaker and stirred until a clear brown solution was obtained.

Example 14

Dry neem seed extract powder (20g) obtained by any one of examples 1 to 6, isopropanol (70g) Igesol, CABS (1.43g) and ethylene oxide condensate, 10mole (8.57g) were taken in a beaker and stirred until a brown homogeneous solution is obtained.

Example 15

Dry neem seed extract powder (20g) obtained by any one of examples 1 to 6, Aromax (60g), cyclohexanone (10g) and Emulsol (172 RH) (10g) were taken in a beaker and stirred for 1h until a clear brown solution was obtained.

The claims defining the invention are as follows:

1. A process for the preparation of azadirachtin, in a dry solid powder form having a purity up to 88% which is useful directly for pesticide formulations, from neem seeds/kernels, which comprises;

(a) disintegrating the neem seeds/kernels into a powder; (b) subjecting the said powder to continuous batch percolation using methanol or aqueous methanol or ethanol or aqueous ethanol at ambient temperature in a column; (c) concentrating the extract and stirring the concentrate with petroleum ether or hexane and phase separating by conventional methods; (d) stirring the denser phase containing major quantity of azadirachtin with a water immiscible organic solvent and water as required depending on the solvent used for extraction and phase separating by conventional methods; (e) concentrating the organic phase and gradually adding the concentrate to petroleum ether or hexane under stirring at ambient temperature (f), filtering under suction and drying under vacuum at a temperature in the range of 25-65°C to obtain a neem seed or kernel extract as a powder containing azadirachtin of 10-19% purity; (g) redissolving the product obtained in step (f) in an organic solvent; (h) adding the solution to petroleum ether or hexane under stirring yielding a white powder, which after filtration and drying under vacuum at 65°C resulting in azadirachtin having 15-26% purity as a white powder (i), dissolving the azadirachtin from step (f) in an organic solvent and subjecting to column chromatography by stepwise elution using different compositions of hexane or petroleum ether and ethyl acetate leading to solid azadirachtin powder of at least 49% and (j) finally dissolving the azadirachtin having up to 49% purity in methanol, ethanol or acetonitrile and subjecting it to HPLC to yield azadirachtin of purity of up to 88% as a solid powder.

2. A process as claimed in claim 1 wherein the disintegration of the neem seeds/ kernels is carried out in a mill to obtain a particle size in the range of BSS-7 (0.2mm) to BSS-72 (2.4mm).

3. A process as claimed in claim 1 or claim 2 wherein the solvent used for extraction of disintegrated neem seeds/kernels in step (b) is methanol, aqueous methanol, ethanol, aqueous ethanol.

4. A process as claimed in claim 3 wherein the solvent used for extraction of disintegrated neem seeds/kernels in step (b) is methanol.

5. A process as claimed in any one of claims 1 to 4 wherein the extract obtained in step (c) is concentrated at atmospheric pressure or under vacuum.

6. A process as claimed in claim 5 wherein the extract obtained in step (c) is concentrated under vacuum.

7. A process as claimed in any one of claims 1 to 6 wherein the organic solvent used in step (d), for separation of azadirachtin is benzene, 2-butanone, chloroform, dichloromethane, dichloroethane, diisopropylether, ethyl acetate, methyltertiarybutylether or toluene.

8. A process as claimed in claim 7 wherein the organic solvent used in step (d), for separation of azadirachtin is ethyl acetate.

9. A process as claimed in any one of claims 1 to 8 wherein the water immiscible organic solvent extract obtained in step (e) is concentrated at atmospheric pressure or under vacuum and in step (f) the second solvent used for preparation of azadirachtin powder is petroleum ether/hexane.

10. A process as claimed in claim 9 wherein the second solvent used for preparation of azadirachtin powder is petroleum ether.

11. A process as claimed in claim 1 wherein the solvent used for redissolution of azadirachtin in step (g), is ethyl acetate or dichloromethane and the solvent used for preparation of azadirachtin powder is petroleum ether/hexane.

12. A process as claimed in claim 1 wherein the solvent used for redissolution of azadirachtin in step (g), is ethyl acetate and the solvent used for preparation of azadirachtin powder is petroleum ether.

13. A process as claimed in claim 1 wherein the stationery phase used for column chromatography is silica gel and the technique used for purification of azadirachtin is stepwise elution employing petroleum ether/hexane, ethyl acetate and their mixtures in step (h).

14. A process as claimed in claim 1 wherein the stationery phase employed is C18 bonded silica gel and the solvent employed for purification of azadirachtin using HPLC is methanol water (6/4) in step (i).

15. A process as claimed in claim 1 wherein the azadirachtin powder obtained in step (f), (g), (h) and (i) is dried in vacuum between 25-65°C for the removal of adhering solvents.

16. A process for the preparation of azadirachtin, in a dry solid powder form having a purity up to 88%, substantially as hereinbefore described with reference to any one of the examples.

17. Azadirachtin, in a dry solid powder form having a purity up to 88% prepared by the process of any one of claims 1 to 16.

18. A neem seed/kernel extract in the form of a dry solid powder having azadirachtin up to 88% which is useful directly for pesticide formulations made by the process of claim 1.

19. A neem seed/kernel extract made by the process of claim 1 in the form of a dry solid powder having azadirachtin up to 88%, substantially as hereinbefore described with reference to any one of the examples.

20. A process for the preparation of emulsifiable concentrate comprising up to 30% by weight of azadirachtin from neem seeds/kernels which comprises stirring the azadirachtin powder having purity up to 88% with solvents or mixtures thereof, emulsifiers or emulsifier continuously with or without synergist and with or without UV stabiliser and thereby obtaining the clear emulsifiable concentrate.

21. A process as claimed in claim 20 wherein the organic solvent used is aromax, 2-butanone, cyclohexanone, dimethylformamide, dimethylphthalate. dioctylphthalate, isobutanol, isobutylmethylketone, isopropanol, solvent C-IX or xylene individually or in suitable combinations.

22. A process as claimed in claim 20 or claim 21 wherein said emulsifiers or emulsifier combinations are commercially available nonionic or ionic emulsifiers.

23. A process as claimed in claim 22 wherein said emulsifiers or emulsifier combinations are Cresolox 3409, Emulsol MAL, Emulsol 172 RH. Igesol. calcium alkyl benzene sulphonate (CABS), ethylene oxide condensates, Triton X 100 or Tween 80.

24. A process as claimed in any one of claims 20 to 23 wherein the piperonyl butoxide is used as a synergist.

25. A process as claimed in any one of claims 20 to 24 wherein the UV stabiliser used is 2-hydroxy-4-octyloxy-benzophenone.

26. A process for the preparation of emulsifiable concentrate comprising up to 30% by weight of azadirachtin from neem seeds/kernels, substantially as hereinbefore described with reference to any one of the examples.

27. An emulsifiable concentrate comprising up to 30% by weight of azadirachtin obtained from neem seeds/kernels, produced by the process of any one of claims 20 to 26.

28. An emulsifiable concentrate prepared by the process of any one of claims 20 to 26 comprising up to 30% by weight of azadirachtin obtained from neem seeds/kernels, which is used as pesticide or in any pesticidal formulations.

29. An emulsifiable concentrate comprising up to 30% by weight of azadirachtin obtained from neem seeds/kernels, substantially as hereinbefore described with reference to any one of the examples.

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