Cropping of the artemisinin (antimalarial drug) yielding *Artemisia annua* cultivars, over a ten year period in the agroclimate of north-west India, has not led to the species becoming a weed

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Abstract

The antimalarial drug artemisinin is extracted from the leaves and flowers, harvested from the naturally growing and cultivated populations, of the asteraceae plant *Artemisia annua*. The species is indigenous to Chongqing region of China where it is a weed and is found growing into extensive wild populations. It has become naturalized (weed) in several agroclimates of its introduction in Asia, Australia, Europe and North and South Americas. *A. annua* is now beginning to be cultivated in several agroclimates of India. Agrotechnologies suitable for cultivating *A. annua* in the Indo-gangetic and North-West Indian plains have been developed and introduced with the farmers. Here, the potential of *A. annua* becoming a weed, in the areas of its cultivation, has been assessed under the New Delhi agroclimatic conditions. Single plants of *A. annua* were observed to produce about 0.14×10^6 viable seeds. Cultivation of *A. annua* in field plots at two locations and enumeration of plants outside the plots of cultivation over a 10 year period was observed not to lead to escape of the plant into areas adjacent to the plots of *A. annua* cultivation. It is concluded that *A. annua*, despite being a prolific producer of seeds, is unlikely to become a weed in the agroclimate of north-west plains region of India. Therefore, subtropical agroclimates such as that of north-west Indian plains offer comparative advantage for the cultivation of the domesticated *A. annua* for artemisinin production.

Keywords: *Artemisia annua* crop, *Artemisia annua* cultivation, *Artemisia annua* cultivars, *Artemisia annua* weed, North-West India agroclimate.

Running title: *Artemisia annua* is not a weed in North West India agroclimate.

Introduction

The aromatic plant species *Artemisia annua*, of the family Asteraceae, is the only natural resource of the secondary metabolite sesquiterpene endoperoxide, artemisinin[1-4]. In this plant, artemisinin is biosynthesized and gets accumulated in the glandular trichomes which occur mostly on the leaves and capitula inflorescences[5-6]. The malaria causing populations of the parasite *Plasmodium falciparum* that have developed genetic resistance against other antimalarials, such as chloroquine and sulphadoxine-pyrimethamine, are sensitive to artemisinin and several of its semi-synthetic derivatives[7-12]. Combinations of artemisinin derivatives, including arteether, artemether, artesunic acid and artelinic acid, with other kinds of selected antimalarials are the only reliable antimalarial therapies available[13-26]. Artemisinin combined with curcumin has synergistic effect against several developmental stages of malarial parasites[27]. Fixed dose artemisinin combinations- artemether + lumafantrine (Coarten), artesunate + amodiaquine (Coarsucam), artesunate + pyronaridine (Pyramax) and dihydroartemisinin + piperaquine (Eurartesin)- are the preferred artemisinin combination therapies (ACTs) for treating malaria as well as for suppressing the development of any kind of artemisinin resistance in the malarial parasite[26-31]. Although some important progress has been made in the discovery of new antimalarial drugs, their possible introduction into antimalarial therapy, as substitute of or in combination of artemisinin, is several years away into future[32-34]. Since synthetic and bioreactor procedures to produce artemisinin and/or its analogues are as yet in the process of development, agricultural production of artemisinin is the essential means to sustain a supply chain of artemisinin[35-38]. To produce 330 tons of artemisinin (or 200 tons of its derivatives), for treating 500 million cases of malaria that occur each year, *A. annua* must be harvested from 10,000 ha of its crops, both cultivated on arable land and found growing naturally in parts of China and other countries[3, 12, 39-44].

Artemisinin and its derivatives are therapeutically important beyond malaria. They are potential drugs against uveal melanoma, oral cancer and angiogenesis of tumors, schistosomiasis, toxoplasmosis, pathogenic fungi and bacteria, including multiple drug resistant tuberculosis, and hepatitis B[45-55]. *A. Annua* has also become an important component of veterinary medicine (56). A steady supply of artemisinin is needed to meet the challenge posed by malaria and also for its other uses, especially as widely used ethnoveterinary medicine[3, 57, 58].

In the immediate future, *A. Annua* cultivation has to become robust to compete with food and vegetable crops. Improved genotypes and optimization of culture and pre- and post- harvest conditions are required for the competitive farming of *A. annua*. The shoot artemisinin content among the genetic resources of *A. annua* is known to vary from < 0.1 to > 2 %[59]. Multifarious approaches are being pursued to obtain genotypes of *A. annua* whose leaves bear high contents of artemisinin. Exercise of recurrent selection in the polycross progeny has led to a population whose members bear on average 1.4 % artemisinin[3, 60, 61 and present study]. Several of the loci that determine the artemisinin content related traits have been mapped[59]; thus efforts for marker assisted selection of *A. annua* genotypes with improved artemisinin yield have begun. The genetic engineering of the elements of the artemisinin biosyntheitic pathway and its regulation is yielding transgenics of *A. annua* that over-produce artemisinin[62, 63]. A crop fertilization schedule that enhances artemisinin yield has been standardized[64]. Several *A. annua* cultivation schedules have been developed for small farmers, in which *A. annua* precedes or follows grain/vegetable crops[3, 20, 65]. A multiharvest/ratoon cropping system of *A. annua* has been developed to obtain artemisinin in high yields (66, 67). Several by-products prepared from residual stems and roots and solvent extracted leaves have been characterized for improving the profit of *A. annua* farmers[68-74]. The stress conditions created pre-harvest by exogenously applied/sprayed salicylic acid, abscissic acid or DMSO and creation of water deficit have been found to significantly increase the artemisinin harvest[75-79]. *A. annua* has begun to be cultivated outside of China and Vietnam, in India, Brazil, Australia, USA, UK, Ghana, Kenya, Madagascar, Mozambique, Nigeria, Tanzania, Uganda and Romania[77]. It's cultivation is likely to be introduced in new areas in these countries and in more countries. *A. annua* is also being harvested from its natural populations abundant in Chongqing region of China and certain other areas of the world with similar agroclimate[64, 77, 80, 81]. There is the possibility that *A. annua* may become a weed when cultivated in areas whose agroclimates match more or less with that in Youyang county, Chongqing, China, thus endangering the practice of general agriculture, in the new areas of *A. annua* cultivation[64, 80]. Since *A. annua* is an alien species for the countries where its cultivation has been introduced, there exist additional dangers if such a species becomes a weed. The alien species growing as weeds can disturb the ecological balance between the local members of the flora and fauna. In several locations of *A. annua* introduction, in Vietnam, Europe, south and north Americas and Australia, the species is seen growing in the wild in the form of natural populations[64, 77].

Some of the important traits shared by annual weeds are – prolific production of viable seeds and efficient seed dispersal. These properties appeared to be present in the *A. annua* genotypes undergoing domestication for cultivation in India. The artemisinin-rich cultivars of *A. annua*, that have been developed for cultivation in the agroclimates of north-west India and Indo-Gangetic plains, are Jeevanraksha, Jeevanraksha-2, Arogya, Jwarharti and Jwarharti-2[3, 60 and present study]. The objective of the present study was to test whether the above Indian cultivars of *A. annua* have the potential of becoming weeds in the agroclimate of North-West India, as represented by New Delhi. This long-term study over a ten year period shows that it is unlikely that the cultivation of *A. annua* in North-West Indian agroclimate will lead to the species becoming a weed of the area.

Material and Methods

Four *A. annua* genotypes (cultivars), namely Jeevanraksha, Jeevanraksha 2, Jwarharti and Jwarharti 2, were used. The cultivars had been developed out of segregants of a cross between two genetically distant accessions[60]. Starting from the F_2 generation, individual segregants were screened at the pre-flowering stage, for artemisinin content in leaves, using a thin layer chromatography procedure (82). Plants bearing > 0.7% artemsinin were selected and rest of the plants were removed. Seeds produced on the selected plants were pooled to raise F_3 generation. This procedure was repeated in each of the subsequent generations, except that plants bearing ≥ 0.9, 1.0, 1.2 and 1.4 % artemisinin were selected in the F4, F5, F6 and later generations. The seeds pooled in F_4, F_5, F_6 and $F_{7/8/9}$ generations were respectively called Jeevanraksha, Jeevanraksha 2, Jwarharti and Jwarharti 2.

The nursery and cultivation procedures have been described earlier[3, 67, 83]. Nurseries were raised in soil + farm yard manure, in clay pots and/or field plots, by sowing of seeds in January-March period. Seedlings were transplanted in field, during the period March-June. The field plots had been applied the N, P, K and S fertilizers @ 80, 40, 40 and 10 kg/ha. Plant to plant, and line to line distances were kept 40 cm and 1 m, respectively. The field plots were irrigated as and when required. The maintenance/cultivation plots of different cultivars were grown in different fields or at a minimal distance of 100 m when grown in the same field.

In the year 2007, the four cultivars were quantitatively assessed for their developmental properties by planting them in the completely randomized design replicated five times. The cultivars were sampled for artemisinin content of leaves at pre-flowering stage in the middle of September and for whole plants in the end of December (Fig. 1). The plants were dug out and kept under a shed for shade drying. Roots, stem, leaves, seeds and leftover capitula were separated and weighed genotype- and replication- wise. The harvest index of seeds was estimated as seed biomass/root + stem + leaves + seeds + rest of the biomass × 100. Seed germination was tested by sowing the seeds in earthen trays filled with soil, using the standrized nursery procedure, in February 2008.

The geographic parameters of New Delhi where these experiments were carried out, a part of the north-west agroclimatic region of India, are: the location 28°38′N 77°12′E; extreme temperatures received range from −0.6 °C (30.9 °F) to 46.7 °C (116.1 °F); annual mean temperature is 25 °C (77 °F); monthly mean temperatures range from 13 °C to 32 °C (56 °F to 90 °F); average annual rainfall is approximately 714 mm (28.1 inches), most of which is during the monsoon rainy season in July-August/September; other seasons are autumn (September-November), winter (November-February), spring (February-March/April) and summer (April-June/July).

Results and Discussion

The average content of artemisinin in young leaves was 0.8±0.2, 1.0±0.2, 1.2±0.1 and 1.4±0.2 in Jeevanraksha, Jeevanraksha 2, Jwarharti and Jwarharti 2 respectively. The inter-cultivar differences in the developmental characteristics were not significant. Therefore, the plant development related observations are summarized collectively in the table 1. *A. annua* was observed to be a profolic producer of seeds. On average basis single plants of *A. annua* produced 13.5 g of seeds which amounted to 6.3% of the total plant biomass. Of the seeds produced, only 56% germinated. Flowering in *A. annua* plants was a continuous process that began in early October and continued until harvest time. Flowering resulted in the production of thousands of capitula (Fig. 1B), each of which bore dozens of flowers. Because of the asynchrony the seeds separated from the harvest must have been at different stages of development. Immaturity was most likely the reason for failure of 44% seeds to germinate. The observations showed that a plant of *A. annua* produced about 0.14×10^6 fertile seeds. The mean weight of a seed being 53 microgram, *A. annua* seeds would get widely dispersed under strong currents of winds. Close planting and multiple harvesting of *A. annua* crop plants has been shown to be a means for large harvests of artemisinin. Seeds produced on two plants of *A. annua* would be more than enough to plant 1 ha crop of the above kind. Thus the entire quantity of seed produced in the present experiment, that occupied less than $250m^2$ of a field plot, would be sufficient to crop *A. annua* over 750 ha of land. A total of 15 such field plots of *A. annua* are estimated to sufficiently meet the annual seed requirements of the crop worldwide (10,000 ha). Apparently, *A. annua*, on account of its tremendous potential to produce fertile seeds could become a weed of the local agroclimate, at New Delhi.

The possibility of *A. annua* becoming a weed of the North-West regional agroclimate of India, represented by the New Delhi environment, was experimentally tested here. The test comprised of enumeration of *A. annua* plants growing in areas of 20m perimeter, and beyond, around the plots *A. annua* cultivation. The yearly surveys for the presence of *A. annua* plants in the perimeters around *A. annua* plots was conducted in late March. Consistent presence of *A. annua* plants outside of the cultivation plots would have meant escape of *A. annua* as a weed, into the local ecological milieu.

One or more cultivars of *A. annua* were cultivated in 1 to 3 field plots at 1 or 2 locations over a 10 year period from 2001 to 2010. The enumeration of *A. annua* escapees in the areas around the field plots of *A. annua* cultivation at the two locations was continued from 2001 to 2011. The cultivation schedule of *A. annua* at the two locations and results of observations on *A. annua* plants seen growing outside the *A. annua* cultivation plots are summarized in the table 2. It will be seen from the table 2 that occasionally a small number of plants were observed to be growing in the 20m perimeter around the plots of cultivation. At the experimental farm site 1 where *A. annua* was cultivated from 2001 to 2005, no *A. annua* plant was observed around the cultivation plots from 2007 to 2011. Like-wise, the perimeters of the field plots 2 and 3 at the experimental farm site 2 that were cultivated with *A. annua* in 2005 and 2006 were observed to be completely free of *A. annua* from 2006 to 2011. The perimeter of the field plot 1 at the farm site 2 where *A. annua* was in cultivation from 2005 to 2010 was free of *A. annua* in 2010 and 2011. No *A. annua* plants were observed around and upto 200m beyond the *A. annua* cultivation plots when such a survey was conducted in 2010 and 2011. Since *A. annua* failed to escape into the environment immediately adjacent to the plots of its cultivation, it is concluded that *A. annua* is unlikely to become a weed in the agroclimate of New Delhi or North-West agricultural region of India.

The World Health Organization led programmes aim to control malaria as early as possible with the use of ACTs[12, 29, 30, 41]. Beside China and Vietnam, the convential producers of artemisinin, several countries, including India, are emerging as suppliers of artemisinin or dried leaves of *A. annua* (the raw material for artemsinin extraction)[77, 84]. India can become a major producer of artemisinin because of the following motivational factors. (a) Semitemperate agroclimates of North-West India and Indo-Gangetic plains are highly suitable for the cultivation of *A. annua*[3, 20, 67]. (b) *A. annua* is unlikely to become a weed in these agroclimates (present study). (c) Indigenously developed agro- and processing- technologies are already available[3, 20, 67, 83-90]. (d) Such internationally developed technologies are also accessible[58]. (e) *A. annua* can be easily fitted into the conventional cropping schedules pravelent in the targeted agroclimates[3, 30, 58, 61]. The comparative advantages of *A. annua* cultivation and processing in India need to be exploited increasingly in the immediate future for benefit of farmers, industry and malaria afflicted populations. The *A. annua* enterprise for artemisinin production requires coming together of the government agencies, industry and farmers and their support groups.

Acknowledgements

Grateful thanks are due to Council of Scientific and Industrial Research (CSIR) and Indian National Science Academy for grant of scientistships to SK and to the Director NIPGR for providing the research facilities. Thanks are due to S.K. Rai, Sunil Kumar and Vinod Kumar for their assistance in field work.

References

1. Klayman, D.L. (1985) *Science* **228** : 1049.

2. Enserink, M. (2005) *Science* **307** : 333.

3. Kumar, S. (2005) *Natl. Acad. Sci. Lett.* **28** : 325.

4. White, N.J. (2008) *Science* **320** : 330.

5. Olsson, M.E., Olofsson, L.M., Lindahl, A.L., Lundgren, A., Brodelius, M.& Brodelius, P.E. (2009) *Phytochem.* **70** : 1123.

6. Arsenault, P.R., Vail, D., Wobbe, K.K., Erickson, K. & Weathers, P.J. (2010) *Plant Physiol.* **154** : 958.

7. White, N.J. (1999) *Phil. Trans. R. Soc. Lond. Ser. B.* **354** : 739.

8. Trape, J.F. (2001) *Am. J. Trop. Med. Hyg.* **64** : 12.

9. Olliaro, P.L. & Taylor, W.R.J. (2003) *J. Exp. Biol.* **206** : 3753.

10. WHO, Access to antimalarial medicines: Improving the affordablility and financing of artemisinin-based combination therapies. (2003)WHO document.

11. Yeung, S., Pongtavornpinyo, W., Hastings, I.M., Mills, A.J. & White, N.J. (2004) *Am. J. Trop. Med. Hyg.,* **71** : 179.

12. Greenwood, B.M., Fidock, D.A., Kyle, D.E., Kappe, S.H., Alonso, P.L., Collins, F.H. & Duffy, P.E. (2008) *J. Clin. Inves.* **118** : 1266.

13. Jefford, C.W. (2001) *Curr. Med. Chem.* **8** : 1803.

14. Olliaro, P.L., Haynes, R.K., Meunier, B. & Yuthavong, Y. (2001) *Trends Parasitol.* **17** : 122.

15. Meshnick, S.R. (2002) *Int. J. Parasitol.,* **32** : 1655.

16. Kannan, R., Sahal, D. & Chauhan, V.S. (2002) *Chem. Biol.* **9** : 321.

17. Ralph, S.A., van Dooren, G.G., Waller, R.F., Crawford, M.J., Fraunholz, M.J., Foth, B.J., Tonkin, C.J., Roos, D.S. & McFadden, G.I. (2004) *Nature Rev.* **2** : 203.

18. Eckstein-Ludwig, U., Webb, R.J., Van Goethem, I.D., East, J.M., Lee, A.G., Kimura, M., O'Neill, P.M., Bray, P.G., Ward, S.A. & Krishna, S. (2003) *Nature* **424** : 957.

19. Kumar, N. & Zheng, H. (1990) *Parasitol. Res.* **76** : 214.

20. Kumar, S. & Srivastava, S. (2005) *Curr. Sci.* **89** : 1097.

21. Ogutu, B.R., Smoak, B.L., Nduati, R.W., Mbori-Ngacha,D.A., Mwathe, F. & Shanks, G.D. (2000) *Trans. R. Soc. Trop. Med. Hyg.* **94** : 83.

22. Ringwald, P., Bickii, J. & Basco, L. (1996) *Lancet* **347** : 24.

23. van Vugt, M., Looareesuwan, S., Wilairatana, P., McGready, R., Villegas, L., Gathmann, I., Mull, R., Brockman, A., White, N.J., Nosten, F. (2000) *Trans. R.. Soc. Trop. Med. Hyg.* **94** : 545.

24. Adjuik, M., Agnamey, P., Babiker, A., Borrmann, S., Brasseur, P., Cisse, M., Cobelens, F., Diallo, S., Faucher, J.F., Garner, P., Gikunda, S., Kremsner, P.G., Krishna, S., Lell, B., Loolpapit, M., Matsiegui, P.B., Missinou, M.A., Mwanza, J., Ntoumi, F., Olliaro, P., Osimbo, P., Rezbach, P., Some, E. & Taylor, W.R. (2002) *Lancet* **20 :** 359.

25. Denis, M.B., Davis, T.M., Hewitt, S., Incardona, S., Nimol, K., Fandeur, T., Poravuth, Y., Lim, C. & Socheat, D. (2002) *Clin. Infect. Dis.* **35**:1469.

26. Marquiño, W., Huilca, M., Calampa, C., Falconí, E., Cabezas, C., Naupay, R., Ruebush, T.K. 2nd (2003) *Am. J. Trop. Med. Hyg.* **68** : 608.

27. Nandkumar, D.N., Nafraj, V.A., Vathsala, P.G. Rangarajan, P. & Padmanahan, G. (2006) *Antimicrob. Agents Chemother.* **50** : 1839.

28. "Saving lives, buying time. Economics of malaria drugs in an age of resistance". National Academy of Sciences (2004), www.nap.edu/catalog/11017.html. Global Malaria Action Plan. Report of the 2008 Artemisinin conference. York. UK.

29. Korenromp, E., Miller, J., Nahlen, B., Wardlaw, T., & Young, M. (2005) World Health Organization (WHO), Roll Back Malaria Partnership, Geneva.

30. World Malaria Report (2008) World Health Organization; http://apps.who.int/malaria/wmr 2008/malaria2008.pdf.

31. Dondorp, A.M., Yeung, S., White, L., Nguon, C., Day, N.P., Socheat, D. & von Seidlein, L. (2010) *Nat. Rev. Microbiol.* **8** : 272.

32. Rottmann, M., McNamara, C., Yeung, B.K., Lee, M.C., Zou, B., Russell, B., Seitz, P., Plouffe, D.M., Dharia, N.V., Tan, J., Cohen, S.B., Spencer, K.R., González-Páez, G.E., Lakshminarayana, S.B., Goh, A., Suwanarusk, R., Jegla, T., Schmitt, E.K., Beck, H.P., Brun, R., Nosten, F., Renia, L., Dartois, V., Keller, T.H., Fidock, D.A., Winzeler, E.A. & Diagana, T.T. (2010) *Science* **329** : 1175.

33. Guiguemde, W.A., Shelat, A.A., Bouck, D., Duffy, S., Crowther, G.J., Davis, P.H., Smithson, D.C., Connelly, M., Clark, J., Zhu, F., Jiménez-Díaz, M.B., Martinez, M.S., Wilson, E.B., Tripathi, A.K., Gut, J., Sharlow, E.R., Bathurst, I., El Mazouni, F., Fowble, J.W., Forquer, I., McGinley, P.L., Castro, S., Angulo-Barturen, I., Ferrer, S., Rosenthal, P.J., Derisi, J.L., Sullivan, D.J., Lazo, J.S., Roos, D.S., Riscoe, M.K., Phillips, M.A., Rathod, P.K., Van Voorhis. W.C., Avery, V.M. & Guy, R.K. (2010) *Nature* **465** : 311.

34. Gamo, F.J., Sanz, L.M., Vidal, J., de Cozar, C., Alvarez, E., Lavandera, J.L., Vanderwall, D.E., Green, D.V., Kumar, V., Hasan, S., Brown, J.R., Peishoff, C.E., Cardon, L.R. & Garcia-Bustos, J.F. (2010) *Nature* **465** : 305.

35. Avery, M.A., Chang, W.K.M. & Jennings-White, C. (1992) *J. Am. Chem. Soc.* **114** : 974.

36. Yadav, J.S., Satheesh Babu, R. & Sabitha, G. (2003) *Tetrahedron Lett.* **44** : 387.

37. Vennerstrom, J.L., Arbe-Barnes, S., Brun, R., Charman, S.A., Chiu, F.C., Chollet, J., Dong, Y., Dorn, A., Hunziker, D., Matile, H., McIntosh, K., Padmanilayam, M., Santo Tomas, J., Scheurer, C, Scorneaux, B., Tang, Y., Urwyler, H., Wittlin, S. & Charman, W.N. (2004) *Nature* **430** : 900.

38. Ro, D.K., Paradise, E.M., Ouellet, M., Fisher, K.J., Newman, K.L., Ndungu, J.M., Ho, K.A., Eachus, R.A., Ham, T.S., Kirby, J., Chang, M.C., Withers, S.T., Shiba, Y., Sarpong, R. & Keasling, J.D. (2006) *Nature* **440** : 940.

39. Hay, S.I., Guerra, C.A., Tatem, A.J., Noor, A.M. & Snow, R.W. (2004) *Lancet Infect. Dis.* **4** : 327.

40. Snow, R.W., Guerra, C.A., Noor, A.M., Myint, H.Y. & Hay, S.I. (2005) *Nature* **434** : 214.

41. WHO, UNICEF, (2005) World Malaria Report-2005; section II: Malaria Burden. http://www.rum.who.int/wmr 2005/html/1-2.htm

42. Kindermans, J-M., Pilloy, J., Olliaro, P. & Gomes, M. (2007) *Malaria J.* **6** : 125.

43. Krishna, S., Bustamante, L., Heynes, R.K. & Staines, H.M. (2008) *Pharmacol. Sci.* **29** : 520.

44. Wells, T.N.C., Alonso, P.L. & Gutteridge, W.E. (2009) *Nat. Rev. Drug Discov.* **8** : 879.

45. Kumar, T.R.S., Khanuja, S.P.S., Jain, D.C., Srivastava, S., Bhattacharya, A.K., Sharma, R.P. & Kumar, S. (2000) *Phytother. Res.* **14** : 644.

46. Kumar, S., Khanuja, S.P.S., Kumar, T.R.S., Jain, D.C., Srivastava, S., Bhattacharya, A.K., Saikia, D., Shasany, A.K., Darokar, M.P. & Sharma, R.P. (2000) *US Patent* **6** : 127, 405.

47. Utzinger, J., Xiao, S., N' Grran, E.K., Bergquist, R. & Tanns, M. (2001) *Int. J. Parasitol.* **31** : 1549.

48. Khanuja, S.P.S., Srivastava, S., Kumar, T.R.S., Shasany, A.K., Jain, D.C., Daroker, M.P., Saikia, D. & Kumar, S. (2002) *US patent* **6** : 423, 741.

49. Dell'Eva, R., Pfeffer, U., Vené, R., Anfosso, L., Forlani, A., Albini, A.& Efferth, T. (2004) *Biochem. Pharmacol.* **68** : 2359.

50. Berger, T.G., Dieckmann, D., Efferth, T., Schultz, E.S., Funk, J.O., Baur, A. & Schuler, G. (2005) *Oncol. Rep.* **14** : 1599.

51. Romers, M. R., Efferth, T., Serrans, M.A., Castano, B., Macias, R.I., Briz, O. & Marin, J.J. (2005) *Antiviral Res.* **68** : 25.

52. Jones-Brando, L., D' Angels, J., Posner, G.H. & Yolken, R. (2006) *Antimicrob. Agents Chemother.* **50** : 4226.

53. de Oliveira, T.C., Oliveira Silva, D.A., Rostkowska, C., Bela, S.R., Ferro, E.A.V., Megalhaes, P.M. & Mineo, J.R. (2009) *Exp. Parasitol.* **122** : 233.

54. Nam, W., Tak, J., Ryu, J.K., Jung, M., Yovk, J.J., Kim, H.J. & Cha, I.H. (2007) *Head Neck* **29** : 335.

55. Efferth, T. (2007) *Planta Med.* **75** : 299.

56. Lans, C., Turnar, N., Khan, T., & Brauer, G. (2007) *Canada Vet. Parasitol.* **148** : 325.

57. Yang, S.P., Yang, X., Huang, J.G. & Ding, D.R. (2004) *J. Trop. Subtrop. Bot.* **12** : 189.

58. World Health Organization (2006) WHO monograph on good agricultural and collection practices (GACP) for *Artemisia annua* L. Geneva.

59. Graham, I.A., Besser, K., Blumer, S., Branigan, C.A., Czechowski, T., Elias, L., Guterman, I., Harvey, D., Isaac, P.G., Khan, A.M., Larson, T.R., Li, Y., Pawson, T., Penfield, T., Rae, A.M., Rathbone, D.A., Reid, S., Ross, J., Smallwood, M.F., Segura, V., Townsend, T., Vyas, D., Winzer, T. & Bowles, D. (2010) *Science* **327** : 328.

60. Kumar, S., Banerjee, S., Dwivedi, S., Gupta, M.M., Verma, R.K., Jain, D.C., Khanuja, S.P.S., Mathur, A.K., Bagchi, G.D., Zehra, M., Mehta, V.K., Naqvi, A.A., Paul, S., Ram, G., Ram, M., Saikia, D., Sangwan, R.S., Kumar, T.R.S., Shasany, A.K., Darokar, M.P., Singh, A.K. & Singh, A. (1999) *J. Med. Aromat. Plant Sci.* **21 :** 47.

61. Ferreira, J.F.S., Laughlin, J.C., Delabays, N. & Magalhaes, P.M.D. (2005) *Plant Genet. Resour.* **3** : 206.

62. Feng, L.L., Yang, R-Y., Yang, X-Q., Zeng X-M., Lu, W-J. & Zeng, Q-P. (2009) *Plant Sci.* **177** : 57.

63. Hong, G-J., Hu, W-L., Cehn, X-Y. & Wang, L-J (2009) *Plant Mol. Biol. Rep.* **27** : 334.

64. Davies, M.J., Atkinson., C.J., Bums, C., Wolley, J.G., Hipps, N.A., Arroo, R.R.J., Dungey, N., Robinson, T., Brown, P., Flochart, J., Hill, C., Smith, L. & Bentley, S. (2009) *Ann. Bot.* **104** : 315.

65. Kumar, S., Srivastava, R.K., Singh, A.K., Kalra, A., Tomar, V.K.S., Bansal, R.P. (2001) *Curr. Sci.* **80** : 563.

66. Kumar, **S**., Gupta S.K., Singh, P., Bajpai, P., Gupta M.M, Singh, D., Gupta K., Ram. G, Shasany, A.K. & Sharma, S. (2004) *Ind. Crops Prod.* **19** : 77.

67. Kumar, S., Gupta, S.K., Gupta, M.M., Verma, R.K., Jain, D.C., Shasany, A.K., Darokar, M.P. & Khanuja, S.P.S. (2002) *US patent* 6,393,763.

68. Gupta, S.K., Singh, P., Bajpai, P., Ram, G., Singh, D., Gupta, M.M., Jain, D.C., Khanuja, S.P.S. & Kumar, S. (2002) *Ind. Crops Prod.* 16:217-224.

69. Goel, D., Goel, R., Singh, V., Ali, M., Mallavarapu, G.R. & Kumar, S. (2007) *J. Nat. Med.* **61** : 458.

70. Goel, D., Singh, V., Ali, M., Mallavarapu, G.R. & Kumar, S. (2007) *J. Nat. Med.* **61** : 187.

71. Jain, N., Srivastava. S.K., Aggarwal, K.K., Kumar, S. & Syamasunder, K.V. (2002) *J. Essent. Oil Res.* **14** : 305.

72. Tripathi, A.K., Prajapati, V., Aggarwal, K.K., Khanuja, S.P.S. & Kumar, S. (2000) *J. Econ. Entomol.* **93** : 43.

73. Kiran, U. & Patra, D.D. (2003) *Agric. Ecosyst. Environ.* **94** : 237.

74. Teixeira da Silva, J.A., Yamokura, L., Kaganda, J., Mookdasanit, J., Nhut, D.T. & Afach, G. (2004) *J. Herbs Spices Medicin. Plants* **11** :1.

75. Pu, G-B., Ma, D-M., Chen, J-L., Ma L-Q., Wang, H., Li, G-F., Ye, H-C. & Liu, B-Y. (2009) *Plant Mol. Biol. Rep.* **28** : 1127.

76. Jing, F., Zhang, L., Li, M., Tang, Y., Wang, Y., Wang, Y., Wang, Q., Pan, Q., Wang, G. & Tang, K. (2009) *Biologia* **64** : 319.

77. Ferreira, J.F.S. & Luthria, D.L. (2010) *J. Agric. Food Chem.* **58** : 1691.

78. Marchese, J.A., Ferreira, J.F.S., Rehder, V.L.G., & Rodrigues, O. (2010) *Brazil J. Plant. Physiol.* **22** : 1

79. Mannan, A., Liu, C., Arsenault, P.R., Towler, M.J., Vail, D.R., Lorence, A. & Weathers, P.J. (2010) *Plant Cell Rep.* **29** : 143.

80. Huang, L., Xie, C., Duan, B. & Chen, S. (2010) *Chinese Mal.* **5** : 18.

81. IOWH/MMV (2008) The Artemisinin Enterprise: Exploring New sources of Artemisinin; University of York, York, p49.

82. Gupta, M.M., Jain, D.C., Verma, R.K. & Gupta, A.P. (1996) *J. Medicin. Aroma. Plant Sci.* **18** : 5.

83. Ram, M., Gupta, M.M., Dwivedi, S. & Kumar, S. (1997) *Planta Med.* **63** : 372.

84. Lommen, W.J., Elzinga, S., Verstappen F.W. & Bouwmeester, H.J. (2007) *Plant Med.* **73** : 1133.

85. Jain, D.C., Tandon, S., Bhakuni, R.S., Siddiqe, M.S., Kahol, A.P., Sharma, R.P., Kumar, S., & Bhattacharya, A.K. (1999) *US Patent* 5,955,084.

86. Jain, D.C., Bhakuni, R.S., Gupta, M.M. Sharma, R.P., Kahol, A.P., Dutta, G.P. & Kumar, S. (2000) *Ind. Res. Prod.,* **59** : 1.

87. Jain, D.C., Bhakuni, R.S., Saxena, S., Kumar, S. & Viswakarma, R.A. (2002) *US Patent* 6,346,631.

88. Jain, D.C., Bhakuni, R.S., Sharma, R.P., Kumar S. & Datta, G.P. (2002) *US Patent* 6,362,219.

89. Jain, D.C., Tandon, S., Bhakuni, R.S., Siddiqe, M.S., Kahol, A.P., Sharma, R.P., Kumar, S. & Bhattacharya, A.K. (1999) *US Patent* 188,008.

90. Kumar, S., Gupta, S.K., Singh, D., Gupta, M.M., Jain C.J., Kahol, A.P., Khanuja, S.P.S. & Ram, G. (2004) *US Patent* 6,685,972.

Legend to figure

Fig. 1- Field views of *Artemisia annua* at the pre-flowering (A) and flowering stages (B) of development.

Table 1- Seed production parameters of single plants, in the artemisinin rich cultivars Jeevanraksha, Jeevanraksha-2, Jwarharti and Jwarharti-2 of *Artemisia annua*

Unit plant parameter[a]	Range of expression		Mean
	Minimum	Maximum	
Shoot biomass[b] (g)	113	259	181±38
Root biomass (g)	19	45	30±7
Seed biomass (g)	7.1	23.8	13.5±5.0
Weight of 1000 seeds (mg)	46	62	53±4
Seed fertility (%)	42	75	56±6
Seed harvest index (%)	3.8	10.3	6.3±0.6

a = observations taken on 10 plants/genotype in the year 2007;
b = seed biomass + stem + leaves + leftover capitula.

Table 2- The land area cropped with different cultivars of *Artemisia annua* at the two sites of the NIPGR experimental farm and the number of *A. annua* plants observed growing outside, in the 20m perimeter, of the cropped field plots, during the 2001 to 2011 period of experimentation

Serial no.	Item/Detail	Experimental farm site 1			Experimental farm site 2		
		Field plot			Field plot		
		1	2	3	1	2	3
1	Total farm area (m^2)	2600	2504	400	3570	3816	3820
2	Area cropped with *Artemisia annua*, in the year 2001;	1000[a]	0	0	0	0	0
3	2002;	200[a]	0	100[c]	0	0	0
4	2003;	400[a]	100[b]	0	0	0	0
5	2004;	200[a]	400[b]	0	400[a]	400[a]	400[a]
6	2005;	200[a]	100[b]	100[c]	400[a]	400[a]	400[a]
7	2006;	0	0	0	400[d, e]	0	0
8	2007;	0	0	0	250[a, b, d, e]	0	0
9	2008;	0	0	0	200[e]	0	0
10	2009;	0	0	0	100[e]	0	0
11	2010.	0	0	0	100[e]	0	0
12	Number of *Artemisia annua* plants found outside the field plot, in the 20m wide perimeter, in the year 2001;	0	0	0	NA[f]	NA	NA
13	2002;	5	0	3	NA	NA	NA
14	2003;	4	0	2	NA	NA	NA
15	2004;	1	2	2	0	0	0
16	2005;	10	6	3	8	9	10
17	2006;	11	0	2	5	0	0
18	2007;	3	0	0	3	0	0
19	2008;	0	0	0	8	0	0
20	2009;	0	0	0	2	0	0
21	2010;	0	0	0	0	0	0
22	2011.	0	0	0	0	0	0

a = Area cropped with the cultivar Jeevanraksha; b = Area cropped with the cultivar Suraksha; c = Area cropped with the cultivar Jeevanraksha-2; d = Area cropped with the cultivar Jwarharti; e = Area cropped with the cultivar Jwarharti-2; f = NA, not applicable.