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Research Article

Herbal Pesticide

Novel Formulation of Herbal Pesticide for Rice Cultivation

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Abstract: The irrational use of chemical pesticides has caused serious health hazards and environmental problems in developing countries including India. Hence, the Present investigation was carried out to formulate an effective Herbal pesticide against three major pests of rice like stem borers, leafhoppers and plant hoppers and to find solution for control of pest population by traditional agricultural practices. Oil of *Azadirachta indica, Vitex nigundo, Ipomoea carnea, Adhatoda zeylanica* and natural surfactant are the main constituents used in the formulations. Larvae of stem borers in the infected stem and hopper damaged part of the plant were selected for the study. A total of six herbal formulations at four different concentrations were sprayed on larvae of stem borers and hopper damaged parts of plants. The developed herbal pesticides have been validated in the field as well as in lab conditions and larval mortality rates were observed. After treatment, the population of pests decreased gradually and the mortality rate of larvae increased. Herbal pesticides focus on providing solutions and relief from various types of insects that cause massive damages to the plants being grown on a farm. These products were made of natural materials that have properties of killing or keeping out pests within the range of the field. The results showed that the extent of growth of these pests was less when compared to control and minimum occurrence of pest attack and Percent Dead Heart (DH) was confirmed in response to herbal formulations. The foremost benefit of this herbal pesticide is managing the Yellow Stem Borer (YSB) pest population and providing healthier cropping systems. Hence it is recommended to use this herbal pesticide for disease free sustainable cultivation of organic rice.

Keywords: Herbal pesticide, Oil extract, Stem borers, Leafhoppers, Percent Dead Heart, Larval Mortality.

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1. INTRODUCTION

Rice (*Oryza sativa* L.), the grain of life and staple food is one of the most important crops in the world, providing food for nearly half of the global population.[1,2] Globally rice is cultivated over an area of about 163.19 million hectare with an annual production of about 719.3 million tonnes. In India rice is cultivated over an area of 42.5 million hectare with a productivity of 3507 kg/ha).[3] The crop is also the most important staple food for about 65% of the Indian population. The rice plant is attacked by more than 100 species of insects, 20 species of them can cause economic damage.[4] Stem borers are the most damaging pest species in Asia until the 1960s,[5,6] and are still causing substantial yield loss.[7] The yield loss may range from 1-20% and may even reach 30-100 % during outbreak condition. The crop is damaged from seedling stage till harvest and cause complete loss of affected tillers.[8] The increased application of chemical pesticides can lead to excess runoff resulting in pollution of water bodies and eutrophication.[9] High tillage practices can reduce soil organic matter leading to desertification[10] and the monocultures and pesticide application associated with modern intensive agricultural methods have resulted in widespread reductions in biodiversity. [10,11] These negative effects can render such intensive agricultural systems unsustainable. Increased use of pesticides has helped to increase the rice yields, but also proved to be unsustainable and cost ineffective due to negative effects of pesticide use on human health and environment. *Scirpophaga incertulas* Walker is the dominant species causing heavy yield losses in the range of 21- 51%. It is a widely distributed monophagous pest and infests the crop from seedling to maturity.[12] The adults of *S. incertulas* are nocturnal, positively phototropic and strong fliers. The moths lay eggs in mass near the tip of the leaf blade of rice usually containing 50-80 eggs. A single female is capable of laying 100-200 eggs. The eggs are covered with pale - orange brown hairs from the anal tufts of the female moths. The eggs laid on the leaf of rice within a field are generally randomly distributed.[13] Leaf hoppers are one of the most abundant groups of plant feeding insects in the world, with leaf hopper and plant hopper species outnumbering that of all species of birds, mammals, reptiles, and amphibians combined. Many species are host specific with their names indicating their preferred host; e.g. rose leaf hopper, grape leaf hopper, potato leaf hopper, etc. Leaf hoppers are wedge shaped and vary in color depending on species. Their name refers leaf hopper's affinity for hopping off of leaf surfaces when disturbed. Plant extracts, because of their natural origin, are biodegradable and they do not leave toxic residues or byproducts to accumulate in the environment. Several plants have several hundred years of history and the non-toxicity at least at oral level is proved. Further, the research and development cost of botanical pesticides from discovery to marketing is much less compared to chemical pesticides. Therefore, plant based products have gained worldwide attention. They are not only effective against crop pests but also safer to natural enemies. Therefore, an attempt was made to evaluate the effectiveness of the plant products to control the pest population of paddy to ensure environmental protection as well as successive farming.

2. MATERIALS AND METHODS

Effect of certain botanical oil pesticides against paddy stem borer, *Scirpophaga incertulas* and leaf hopper of rice under greenhouse conditions was tested as suggested by Vennila, 2012.[13] These trial experiments were undertaken in the PG and Research Department of Microbiology, S.T.E.T. Women's College, Sundarakkottai, Mannargudi , Tamil nadu , India.

2.1 Preparation of herbal oil pesticide

Seven locally available plant species were selected for preparation of pesticide. Leaves and seeds of the plants have been used for oil extraction.

2.2 Preparation of Neem fixed oil

The ripened fruits of *Azadirachta indica* were collected and fruit coat and pulp was removed to obtain the seeds. The seeds were dried at the temperature of 50˚C to 60˚C for 48 to 72 hours to remove the moisture content. The fixed Oil was extracted by cold press technology and filtered to remove the impurities.[14]

2.3 Preparation of Vitex negundo volatile oil

Hydro-distillation of macerated mature leaves of *Vitex negundo* (chaste tree) was carried out for 16 to 18 hours at 65˚ C to 70˚C. The volatile oil was obtained after distillation process and double filtration was performed to remove the impurities and used for the formulation of herbal pesticide. [14]

2.4 Preparation of Ipomoea carnea volatile oil

Matured leaves of *Ipomoea carnea* were collected and dried at temperature between 50˚ C - 70˚ C for 3-4 days. Then the plant material macerated and steam distilled, where the essential oils passed into distillate from which they were extracted by pure organic volatile solvents.

2.5 Preparation of Adhatoda zeylanica volatile oil

The matured leaves of *Adhatoda zeylanica* were dried at temperature between 50˚C - 65˚C for 3-4 days. Then the plant material macerated and steam distilled, where the essential oils passed into distillate from which they were extracted by pure organic volatile solvents.

2.6 Formulation of herbal Pesticide

The pesticide formulation was prepared by adding equal amounts (10 ml) of each oil in a sequential manner in a stainless steel vessel. Oil was poured in the vessel at room temperature under continuous stirring condition and mixing was continued for 30 minutes at 1500 rpm. Finally, the emulsifier PEG was added to the above mixture and stirred for 60 minutes. The prepared formulation was left undisturbed to observe any sedimentation.[15]

2.7 Evaluation of herbal pesticide in rice

Effect of each oil extract was tested through spray treatment in individual and combined form separately in pots containing rice plants at the stage of tillering. There were 6 treatments (Table 1)

Table 1- Evaluation of herbal pesticide in rice

Treatment	Components	Concentration (in ml)
T1	Fixed oil of *Azadirachta indica*	25
T2	Volatile oil of *Vitex negundo*	25
T3	Volatile oil of *Ipomoea carnea*	25
T4	Volatile oil of *Adhatoda zeylanica*	25
T5	*Azadirachta indica* + *Vitex negundo* + *Ipomoea carnea* + *Adhatoda zeylanica* + Emulsifier	10 (Each)
T6	Control (Water spray)	25

Pots of diameter 20x20 cm were used for the experiment in the seedling stage. The pots were filled each with 2 kg of clay loam soil to which 500 g of Farm Yard Manure was applied and mixed. The medium duration paddy variety ADT 43 was used for the studies. Fresh seeds of *Oryza sativa* ADT 43 were procured from Tamil Nadu rice Research Institute (TNRRI), Aduthurai, Tamilnadu and used for this study. The healthy seeds were sown into soil with recommended nutrients and collected for the study at the stage of tillering and transplanted into the pots. The pots were watered using rose water can at an interval of 2 days.[15] The pots were used for studying the relative toxicity of insecticides against adult moths and efficacy of insecticides against neonate larvae at tiller stage.

2.8 Release of Adult Moths on Transplanted Pots

The selected pesticides were sprayed on plants at 150 DAT (4 hills/pot) and later covered with a thin polythene cover and three adult moths were released on pesticide sprayed plants[16] and each treatment was replicated thrice.

2.9 Release of Neonate Larvae on Transplanted Pots

Recommended quantities of selected pesticides were sprayed on to plants at 15 DAT (4 hills/pot) and covered with a polythene cover. One day, after pesticide application 4 neonate larvae were released per pot (1 neonate larvae per hill) using brush into leaf whorl. Untreated control (water spray) served as check and each treatment was replicated thrice.[16]

2.10 Observations of Survival of Moths and Dead Hearts

Effect of pesticides on the adult moths was recorded by monitoring the survival of the moths and number of egg masses laid at 24 hrs interval and by means of egg hatchability. Each day the survival of moth and egg mass laid by moths was counted. The damage by neonate larvae in the vegetative phase manifests as death of the growing shoot known as 'dead heart'. The number of dead hearts was recorded at 5, 10 and 15 day after release of neonate larvae. The per cent dead heart (DH) was calculated by using the following formula suggested by Vennila, 2012.[13]

$$\text{Dead Heart (\%)} = \frac{\text{Number of dead hearts}}{\text{Total tillers}} \times 100$$

2.11 Plant growth Measurements

By measuring plant growth, the effect of herbal pesticides for plant growth can be evaluated and it is helpful to calculate correlation between plant growth and Dead Heart formed by pest attack. From shoot and root length, percent phytotoxicity was calculated using the following formula to find whether any toxic effect of added pesticides is observed over plant growth suggested by Abdul Baki, 1973. [17,18]

$$\text{Phytotoxicity (\%)} = \frac{\text{Shoot/Root length in control} - \text{Shoot/ Root length in treatment}}{\text{Shoot/Root length in control}} \times 100$$

2.1 Evaluation of herbal pesticides on leaf and plant hopper

Green leaf hoppers are the most common leaf hoppers in rice fields and are primarily critical because they spread the viral disease tungro. Green leaf hoppers are common in rain fed and irrigated wetland environments. They are not prevalent in upland rice. Both the nymphs and adults feed on the dorsal surface of the leaf blades rather than the ventral surface. They prefer to feed on the lateral leaves rather than the leaf sheaths and the middle leaves. They also prefer rice plants that have been fertilized with large amounts of nitrogen.[14] The selected herbal pesticides were sprayed into a hopper damaged part of the plant in the paddy field. Hopper damage part was assessed by observation of symptoms produced by the virus in the leaf of the young plants and their control towards herbal pesticide formulation treatment were assessed with control plants.

3. STATISTICAL ANALYSIS

Mean was calculated to facilitate the comparison of the data of various growth parameters in all samples. The student's t' test was carried out to find whether the differences between samples are significant or not. Limits of mean can be calculated by relating the standard deviation of the samples to probability of deviation from the population mean. In other words, the sample mean is related to the population mean by means of a confidence interval. In such a case, a value of 't' and also a desired level of significance is required.

4. RESULTS AND DISCUSSION

4.1 Effect of Pesticides on Adult Moth Survival

Survival of female moths at 2 DAR showed that all the treatments except *Ipomea carnoea* recorded lower survival of moths (33.33%). At the end of 3 days, the average number of moths alive in the various treatments revealed that all the treatments showed significant response in control of pest population over control (66.66%) (Table-1). From the results, it is clearly indicated that herbal pesticides had significantly reduced the survival of the moths.

4.2 Effect of Pesticides on Larval Damage

There is a significant reduction in larval damage and dead heart appearance were observed over control. At 10 DAR, no Dead Heart damage was found in mixed botanical oil and *Azadirachta indica*, whereas in control, 26.5 % Dead Heart damage was found. At 15 DAR, the lowest DH was recorded in Mixed botanical oil (10.5 %) followed by *Azadirachta indica* (12.5 %), *Adhatoda zeylanica* (14.3%). From the results, it is concluded that the damage in all treated plants had increased at a slow rate from 5 DAR to 15 DAR and no further increase was observed (Table-2). This indicates that there are no live larvae to cause damage. Thus, the studies clearly revealed that egg hatching percent was lower in all the treatments as compared to control. The herbal pesticides proved to be effective in inhibiting egg hatching irrespective of the crop stage. Also all the herbal extracts proved effective in reducing the adult survival and also significantly reduced the egg laying. The reason attributed for the less larval hatchability could be that, either unviable eggs were laid on the plant or ovicidal action due to penetration of herbal insecticides through the egg shell. Tomizawa *et al.* (1974)[20] reported that when insecticide is applied as spray, the effective concentration remains in the plant for a longer period. This explains the possibility of penetration of insecticide through egg shells and also Richards et al.,(1979)[21] reported that nicotinoid insecticides penetrated into egg shells through micropyles .

4.3 Plant growth Measurement

The plant growth has significantly increased in all treated pots compared to control. Shoot and root length measurements were taken and percent phytotoxicity were calculated. The results showed that there is no phytotoxicity observed in growth of plants. Negative values were obtained in percent phytotoxicity and this negative value showed the positive outcome of added herbal formulation on plant growth (Table-3 & Table 4). From these results, it is concluded that the pesticide spray is helpful in the growth of plants. The results of the present study on the incidence of stem borer was similar with the results of Deol *et al.,* (2001)[22] who reported that chlorophyll loss increased gradually as the duration of the feeding period increased in wheat aphids, *Diuraphis noxia* (Mordvilko) and greenbugs, *Schizaphis graminum* (Rondani) *i.e.*, up to 10 days. However, for greenbugs, chlorophyll loss increased more quickly upto the 4th day of feeding, and then remained relatively constant. Girma *et al.,* (1998)[23] opined that chlorophyll loss continued with increase in feeding of aphids, Schizaphis graminum in sorghum.

4.4 Effect of herbal pesticides on leaf and plant hopper

Apart from pesticidal activity against YSB moths and larvae, other pest control effects and microbial disease control ability of this herbal product were monitored. The presence of most common harmful pests of paddy like Green hopper, brown planthopper, white backed planthopper, leaf folder case worm and gundhi bugs were observed continuously. The presence of common microbial diseases of paddy like blast, brown spot, bacterial leaf blight, sheath blight and sheath rot were carefully observed in the plant to analyze the effect of these herbal pesticides on disease control. The outcome showed a satisfied control effect in both pest attack and microbial diseases compared to untreated plants. Reduced microbial infection was observed in pesticide treated fields and it showed the effect of these herbal formulations on leaf and plant hopper which acts as a vehicle for transmission of microbial diseases. From this study, it is recommended to use this herbal formulation for controlling all major pests and microbial diseases of rice in a safe method. Excessive use of chemical pesticide creates many serious threats such as elimination of beneficial predators and insects, depletion in soil microbial diversity, resistance among the pests and diseases, deposition of toxic residues, etc.[24] Similar were the reports of Korsak and Sato (1977)[25] , where they found that the chemical pesticide results in the destruction of various beneficial microbes, flora & fauna and also causes serious diseases in human. Therefore, there is a great need to develop green and cheaper alternatives for handling economically important pests. The developed formulations are appropriate for farmers who engage in organic as well as conventional farming. However, there are also some general challenges with the use of herbal pesticides. They tend to be more slow-acting and may be very specific to the life cycle of the pest. Other attributes such as persistence in the environment have both a benefit and challenge that must be balanced.[26] Intensive use of nitrogenous fertilizers, high yielding varieties, sequential cropping and indiscriminate use of insecticides has resulted in various insect pest problems in rice crops. Of the 100 or more species of insects known to attack rice crop, Yellow Stem Borer (YSB), *Scirpophaga incertulas* (Walker) is the dominant species causing severe yield losses in the rice crop at the range of 21-51%.[12] The yellow stem borer (YSB) is one of the major pests in all rice growing regions of India causing heavy yield losses.[27] The increased application of chemical pesticides can lead to excess runoff resulting in water pollution and eutrophication.[9] High tillage practices can reduce soil organic matter leading to desertification and the mono cultures and pesticide application associated with modern intensive agricultural methods have resulted in widespread reductions in biodiversity.[10, 11] These negative effects can render such intensive agricultural systems unsustainable. Increased use of pesticides has helped to increase the rice yields, but also proved to be unsustainable and cost ineffective due to negative effects of pesticide use on human health and environment.[28,29,13] There is a growing body of evidence to suggest that in the past 4-5 decades, there has been an excessive dumping of chemical toxins on the soil. As a result, the soil has become barren and ground water toxic, in many places. Contrast this with organic inputs that are safe, non toxic, and low cost successive technology. The organic agriculture improves soil fertility, soil biogeochemical cycles and biological activity which make available to the sustainable agricultural production.[30,31]

Table 1A: Effect of Pesticides on adult moth survival					
S.NO	Average survival of moth	Moth survival (%)			Treatments
		3 DAR	2 DAR	1 DAR	
1	44.44	0	33.33	100	Oil of *Azadirachta indica*
2	44.44	0	33.33	100	Oil of *Vitex negundo*
3	66.66	33.33	66.66	100	Oil of *Ipomoea carnea*
4	44.44	0	33.33	100	Oil of *Adhatoda zeylanica*
5	44.44	0	33.33	100	Mixed Botanical oil
6	88.88	66.66	66.66	100	Control (Water spray)

(a) Each value is mean of 3 replication

Survival of female moths at 2 Day after Release showed that all the treatments except *Ipomea carnoea* recorded lower survival of moths (33.33%). Other treatments *Vitex negundo*, *Adhatoda zeylanica, Azadirachta indica* also showed better results in average survival of the moth (44.44 %) over control (88.88%). overall response revealed that all the treatment showed significant response in control of pest population.

Table 2:Effect of Pesticides on larval damage (DH%)				
	Dead Heart (%)			Treatments
S.NO	15 DAR	10 DAR	5 DAR	
1	26.5	12.5	0.00	Oil of *Azadirachta indica*
2	20.5	14.5	7.5	Oil of *Vitex negundo*
3	25.0	15.5	9.0	Oil of *Ipomoea carnea*
4	25.3	14.3	3.3	Oil of *Adhatoda zeylanica*
5	17.3	10.5	0.00	Mixed Botanical oil
6	71.2	44.2	26.5	Control (Water spray)

(a)E each value is mean of 3 replication

Decreasing larval damage and dead heart appearance were observed over control. At 10 DAR, no Dead Heart damage was noted in mixed botanical oil and *Azadirachta indica*, whereas in control 26.5 % Dead Heart damage was found. At 15 DAR, the lowest DH was recorded in Mixed botanical oil (10.5 %) followed by *Azadirachta indica* (12.5 %), *Adhatoda zeylanica* (14.3%).

Table 3:Growth of tillers at 10 day after release of pest					
	Length of tillers				Treatments
	Larvae released plant		Adult moths released plant		
S.NO	Root length (cm)	Shoot length (cm)	Root length (cm)	Shoot length (cm)	
1	13.8±1.5	38.0±2.6	13.6±1.4	39.1±3.9	Oil of *Azadirachta indica*
2	13.7±1.3	42.5±1.5	13.6±1.5	41.6±2.0	Oil of *Vitex negundo*
3	13.8±0.9	40.5±3.5	13.5±0.5	38.6±4.3	Oil of *Ipomoea carnea*
4	13.8±1.2	36.7±2.3	13.7±1.5	42.3±2.5	Oil of *Adhatoda zeylanica*
5	13.9±1.6	40.1±3.6	13.5±2.0	40.9±3.5	Mixed Botanical oil
6	13.7±1.6	36.3±2.0	13.5±1.3	36.5±2.2	Control (Water spray)

Values are represented as Mean± Standard Deviation (n=5)
P<0.01 when compared with control

The plant growth has significantly increased in all treated pots compared to control. Shoot and root length of the plants in all treatments showed better results. Better growth responses were noted (Root length=13.9±1.6 and Shoot length=40.1±3.6) in the larvae released plant treated with mixed botanical oil followed by *Adhatoda zeylanica* treated plant. In overall, significant plant growth responses were noted in all treatments over control.

Table 4:Phytotoxicity evaluation of tillers at 10 day after release of pest					
	Phytotoxicity (%)				Treatments
S.NO	Larvae released plant	Adult moths released plant			
	Root		Root	Shoot	
1	-0.72	1	-0.74	-7.12	Oil of *Azadirachta indica*
2	0	2	-0.74	-13.97	Oil of *Vitex negundo*
3	-0.72	3	0	-5.75	Oil of *Ipomoea carnea*
4	-0.72	4	-1.48	-15.89	Oil of *Adhatoda zeylanica*
5	-1.45	5	0	-12.05	Mixed Botanical oil
	0	6	0	0	Control (Water spray)

After treatment, Shoot and root length measurements were taken and percent phytotoxicity were calculated in the both larvae and adult moths released by the plant. The results showed that there is no phytotoxicity in plant growth. Negative values were recorded in percent phytotoxicity and this negative value showed the positive outcome of added herbal formulation on plant growth response. It reveals the growth enhancing capability of these herbal formulations. The results showed that the plant treated with mixed herbal oil formulations and Azadirachta indica exhibited high toxicity against larvae upto 15 DAR with absence of Dead Hearts (DH). All the treatments were significantly different from untreated control (71.2 %) and provide better result in reducing dead hearts. The average per cent dead hearts in all treatments revealed that mixed botanicals showed high toxicity by recording lowest % DH (17.3 %) which was significantly differing from untreated control. Thus, the herbal sources were proved to reduce pest damage and percent dead heart in rice. From this evidence, herbal oil pesticide in paddy field is proved for safe and sustainable organic farming. Also over dependence on chemical pesticides has led to replacement of most of the natural enemies of insect pests leading to development of biological vacuum in the existing rice ecosystems. Pest resurgence, pesticide residue on the produce and a widespread increase in the development of insecticide resistance are other inherent contradictions associated with chemical toxicants. [32] After experiencing heavy loss to crop, negative impact on environment, and development of resistant species, planners and researchers have shifted the pest control programme from chemical to biological and ultimately to Integrated Pest Management by traditional practices.[33]

5. CONCLUSION

Plant extracts have assumed special significance in the present day strategy of developing ecologically safe methods of plant disease management. The extract from different plant sources could be used separately or in combination which can result in control of the disease at lower concentration of extracts. Pest management in organic farming is a challenging task without the use of chemical insecticides. It involves careful planning in advance through a slight modification in the cultural practices as primary methods of pest control. The use of environment friendly practices is needed such as use of plant based products as a tool for organic farming principles. Therefore, it was concluded that the selected botanical oils are considered as the best remedy for treating pest populations without disturbing natural diversity of soil. The positive influence of herbal oil formulation in pest and disease control capability was confirmed by this study. The result showed minimum occurrence of pest attack and per cent Dead Heart (DH) in response to herbal oil formulation treatment in the rice cultivation. Hence it is recommended to use this herbal pesticide for disease free sustainable cultivation of organic rice.

6. AUTHORS CONTRIBUTION STATEMENT

Dr. K. Ahilandeswari curated the data and prepared the original draft. Dr. N. Uma Maheswari analyzed these data and provided valuable inputs towards designing of the manuscript. Both authors discussed the methodology, results and contributed to the final manuscript.

7. ACKNOWLEDGEMENT

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8. CONFLICT OF INTEREST

Conflict of interest declared none.

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