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PESTICIDAL EFFICACY OF SELECTED PLANTS OF NEPAL

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ABSTRACT

Plant extract of *Acorus calamus* L, *Adhatoda vasica* L, *Artemisia vulgaris* L, *Calotropis gigantea* (L) W. T. Aiton., *Centella asiatica* (L) Urb., *Curcuma longa* L, *Melia azadirach* L, *Nicotiana tabacum* L, *Datura stramonium* L, *Rhus wallichii* Hook F, *Punica granatum* L, *Vitex nugundo* L, *Wrightia arborea* (Dennst.) Mabb. are studied for insecticidal property against cabbage aphids *Brevicoryne brassicae* L. These plants were also evaluated for potentiality against plant pathogens *Botrytis cinerea*, *Sclerotium rolfsii*, *Fusarium moniliforme*, *Fusarium proliferatum*, *Fusarium oxysporum* and compared with standard fungicide metalaxyl. All screened plants at concentration of 1.25%, 2.5%, 5%, 10% and 20% exhibited selective response with different stain of plant fungi. *Acorus calamus* L, *Nicotiana tabacum* L, *Adhatoda vasica* L, *Artemisia vulgaris* L shows significant control on cabbage aphids. *Nicotiana tabacum* L, showed prominent effect even at lowest concentration ie 1.25% with 50% for aphids. Diluted extracts of *Rhus wallichii* Hook F, *Wrightia arborea* (Dennst.) Mabb., *Curcuma longa* L, *Calotropis gigantea* (L) W. T. Aiton., at 20% concentration were found most effective against plant pathogens *Sclerotium rolfsii*, *Fusarium moniliforme*, *Botrytis cinerea*, *Fusarium oxysporum* respectively.

INTRODUCTION

Organochlorine, organophosphate and carbamate insecticides are of major concern of agriculture because of their toxicity and persistence in the environment. During the past three decades, indiscriminate use of chemical pesticides in agriculture has created serious health and environmental problems in many developing countries. Various studies in Nepal reported the massive use of chemical pesticides in vegetable growing areas that raised issue of possible health risks [1,2]. Pesticide pollution not only affects human health, but also other ecological assets, such as soil surface and ground water, micro and macro flora and fauna [3]. Natural products are an excellent alternative to synthetic pesticides as a means to reduce negative impacts to human health and the environment. Use of products from natural resources and even the extremely biodegradable synthetic and semi synthetic products in pest management, has been considered to constitute the umbrella of green pesticides [4].

Due to the environmental limitations and increasing legal restrictions on the use of many synthetic pesticides, there is a need for the development of alternative control measures that will be cheap, environmental friendly and easily adaptable by local farmers through the abundant flora diversities that exist in Nepal. Studies have reported that more than 90% of the total pesticides are used in vegetable farming in Nepal [5]. Dome of these farmers do not follow the pre-harvest waiting period, apply pesticides near harvesting time, and some farmers even dip vegetables in pesticides before selling [6]. Thus elimination of the insects and pests or mitigation of their activities will go a long way in reducing

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world food crisis as well as improve organic farming. Complete elimination of toxic pesticides would have devastating effects on the farmers. Development of resistance to existing conventional pesticides, the increasing environmental pollution and health dangers created by synthetic pesticides mean that there is a great need for new kinds of useful pest control agents with higher activity against the target pests, and a lower impact on humans and the environment [7]. Therefore it is essential to find out some herbal pesticides as an alternative ways to maintain pesticides that does not risk contaminating water, ecosystems, and people. After several investigations by our research group on Nepalese medicinal plants against human pathogenic microorganisms, the present study was carried out to evaluate on the natural pesticide property of some of these plants by using aphids and plant fungi [8-16]. This treatment may offer the possibility of controlling of different fungal diseases and aphids in Nepalese crops that offers a safe and effective alternative to commercial insecticides for organic farming.

MATERIALS AND METHODS

Plant materials

Plant samples of *Acorus calamus* L, *Nicotiana tabacum* L, *Calotropis gigantea* (L) W. T. Aiton. From Hetauda, *Vitex nugundo* L, *Artemisia vulgaris* L, *Adhatoda vasica* L, *Wrightia arborea* (Dennst.) Mabb. From Arghakhachi, *Melia azadirach* L, *Datura stramonium* L, *Rhus wallichii* Hook F, *Punica granatum* L, *Curcuma longa* L, *Centella asiatica* (L) Urb., from Kavre district of Nepal. Samples were officially identified by Tirtha Maiya Shrestha, faculty of Department of Pharmacy, Kathmandu University and voucher specimen has been deposited in the Department. The collected samples were subjected to air drying, grinded and stored until use.

Test organisms

Different concentration of plant extract (1.25%, 2.5%, 5%, 10%, 20%) were tested for its insecticidal property against *Brevicoryne brassicae* L (cabbage aphids). And these extract solutions were tested for fungicidal property against *Botrytis cinerea*, *Sclerotium rolfsii*, *Fusarium moniliforme*, *Fusarium proliferatum*, *Fusarium oxysporum*. These microorganisms were provided by National Agricultural Research Centre (NARC) Khumaltar, Nepal. The pathogens were kept under refrigeration (4°C) until use.

Extraction

Extraction is done by using methanol in rotary shaker for 48 hours. Extract is then dried on the water bath at temperature 70 ^{0}C. The plant extract were diluted by using DMSO and cow urine for the test on Aphids, and DMSO and distilled water for the test on plant fungi to a dilution series of 1.25%, 2.5%, 5%, 10%, 20% [17,18].

Insecticidal activity

Insecticidal property of plants was tested at cultivated filed of "Everything Organic Nursery", Patlekhet, Kavre, Nepal at 28±2^{0}C during January-March. Three replicated trials were conducted in different plots of the field. Different dilution series (w/v) (1.25%, 2.5%, 5%, 10%, 20%) using DMSO and cow urine were sprayed to the isolated portion of the cabbage containing aphids. Observation is done after 2.5 hours to calculate mortality percentage of aphids on different concentration. The blank solvents i.e. DMSO and cow urine were sprayed as a control. Experiments were carried out based on previous publication with some modification [19].

Antifungal Activity

Disc diffusion method was employed for the screening and determination of antifungal activity of plant extract. The standard suspension of tested microorganism was prepared and spread on the solid media plates with cotton swab. Filter paper discs (6 mm in diameter) were individually impregnated with 1µL of diluted plant aliquots mentioned above. DMSO and distilled water combination was used as negative control and standard fungicide solution was used as positive control. The plates were allowed to stand for 2 hours at room temperature. Media plates were placed on the incubator at 37° C for 48 hours. Each test was performed in four replicates and repeated thrice.

Statistics

Statistical analysis was performed using SPSS 15.0 version. Percentage mortality values for different exposure times were subjected to analysis of variance (one-way ANOVA) using the same statistical program to see significant variation between mortality and concentration; mortality and different plant extracts. Pearson correlation was performed to see the relation between different concentrations of plant extracts and mortality of cabbage aphids.

RESULT AND DISCUSSION

Plant extract of *Acorus calamus* L, *Nicotiana tabacum* L, *Calotropis gigantea* (L) W. T. Aiton., *Vitex nugundo* L, *Artemisia vulgaris* L, *Adhatoda vasica* L, *Wrightia arborea* (Dennst.) Mabb, *Melia azadirach* L, *Datura stramonium* L, *Rhus wallichii* Hook F, *Punica granatum* L, *Curcuma longa* L, *Centella asiatica* (L) Urb., were studied for insecticidal property against cabbage aphids (*Brevicoryne brassicae*). These plants are also evaluated for potentiality against plant pathogens *Botrytis cinerea*, *sclerotium rolfsii*, *Fusarium moniliforme*, *Fusarium proliferatum*, *Fusarium oxysporum*. The increasing dose of plant extracts caused a significant increase in the mortality rate when the aphids were exposed to the samples for 2.5 hours. Percentage mortality of aphids was best shown by *Nicotiana tabacum* L and

Artemisia vulgaris L (Figure 1). The mortality effect of *Adhatoda vasica* L, was very strong than other plant extracts. LC_{99} value for *Nicotiana tabacum* L was found to at 10% of but the LC_{50} value was found to below of 1.25% of the plant extract. LC_{99} value for *Artemisia vulgaris* L was found to at 20% of treatment and the LC_{50} value was found to be 1.25%. While the effect of *Adhatoda vasica* L was found gradually increased by increasing the strength of plant extract. Sigma stat analysis showed that significant positive correlation was seen between mortality of aphids and concentration of different plant extracts ($p<0.01$).Also, one way ANOVA showed significant variation in mortality rate with different plant extracts ($p<0.01$). Therefore the trend showed that, increasing the concentration of extract, percentage mortality of aphids was also increased.

There was no zone of inhibition for negative control with fungi *S. rolfsii*. The plant extracts showed that *S. rolfsii* was little susceptible to Methalaxyl which was used as standard, while susceptibility was gradually decreased in *Botrytis cinerea*, *Fusarium moniliforme*, *Fusarium oxysporum*, and then *Fusarium proliferatum*. The result showed that *S. rolfsii* was intermittently sensitive to *Rhus wallichii* Hook F as compared to others. Samples like *Acorus calamus* L, *Calotropis gigantea* (L) W. T. Aiton., *Centella asiatica* (L) Urb., showed zone of inhibition at 10% and 20 % only against *S. rolfsii. A. calamus* showed zone of inhibition at 20% only and both *Curcuma longa* L and *Calotropis gigantea* (L) W. T. Aiton., showed zone of inhibition even at 10%. *Vitex nugundo* L and *Wrightia arborea* (Dennst.) Mabb showed simultaneous increasing order of zone of inhibition with increasing concentration. *Wrightia arborea* (Dennst.) Mabb has shown effective inhibition even at 2.5 % indicating its strong property than other plants, hence this plant can considered as more effective for future utilization (Figure 2). *Acorus calamus* L showed zone of inhibition only at the 20% (Figure 3).*ß*-asarone from rhizomes of *A. calamus* exhibits insecticidal action against the various insect pests and therefore the results of present study were positively correlated with the finding of previous study [20]. *Curcuma longa* L and *Calotropis gigantea* (L) W. T. Aiton. started their efficacy against *Fusarium moniliforme* at 10% but many of the plants did not shown their effect at lower concentrations of plant extract. Similar result was also found with the fungal strains *Fusarium oxysporu* and *Candida albican* as well as bacterial strains *Staphylococcus aureus*, *Escheriehia coli* in previous finding [21].

All plant extracts except *Centella asiatica* (L) Urb., showed some effect against *Bortytis cinerea* organism (Figure 4). *Curcuma longa* L showed strongest activity even at 10 % concentration in comparison to others. *Rhus wallichii* Hook F showed gradual response on inhibition of *Bortytis cinerea* by increasing the concentration, while it showed strong action even at 1.25 % of concentration. Therefore this plant could be a natural pesticidal agent to control the *Bortytis cinerea*. Similarly, several plants were found active agains the *Fusarium oxysporum* (Figure 5) and *Fusarium proliferatum* (Figure 6) in comparison to standard (Figure 7).

Figure 1. Percentage mortality of cabbage aphids after exposure to four different plant samples.

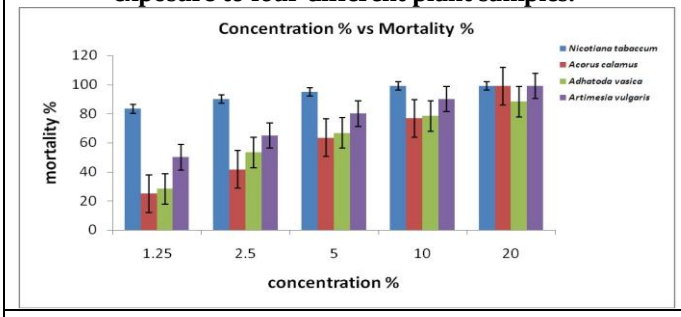


Figure 2. Zone of inhibition of different concentrations of samples on *Sclerotium rolfsii.*

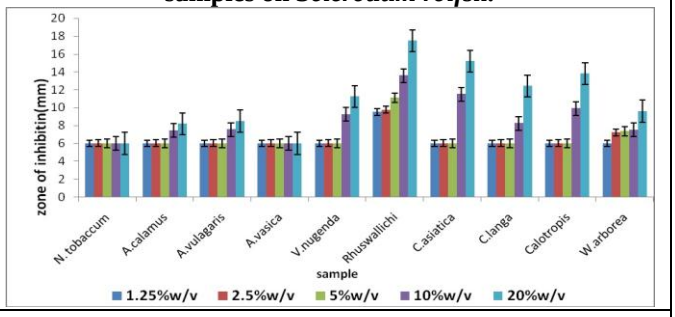


Figure 3. Zone of inhibition of different concentration of samples on *Fusarium moniliforme.*

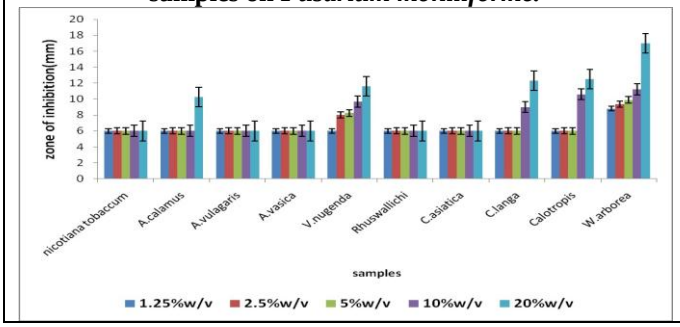


Figure 4. Zone of inhibition of different concentration of samples on *Bortytis cinerea.*

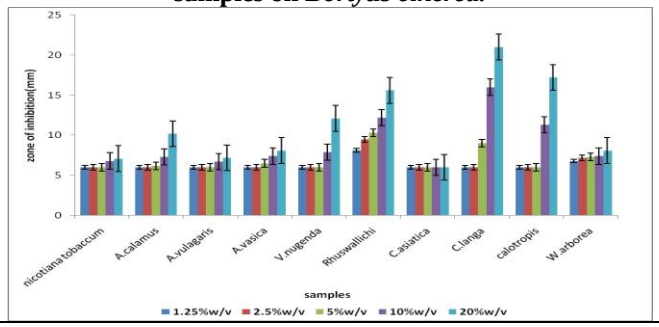


Figure 5. Zone of inhibition of different concentration of samples on *Fusarium oxysporum*.

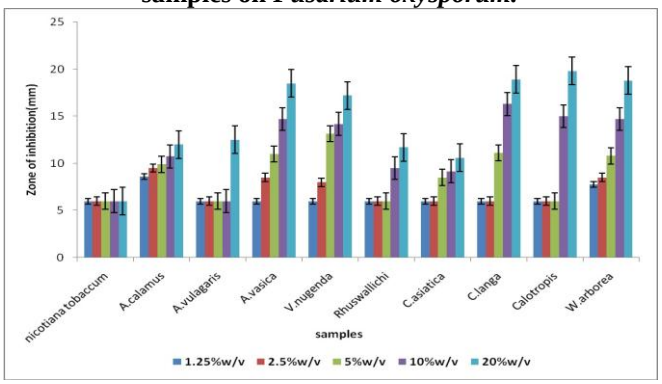


Figure 6. Zone of inhibition of different concentration of samples on *Fusarium proliferatum*.

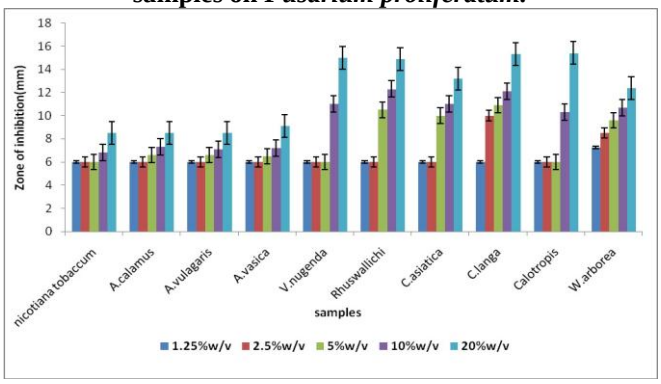
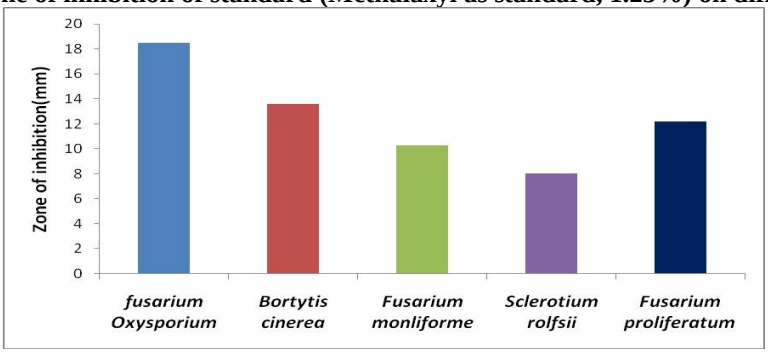


Figure 7. Zone of inhibition of standard (Methalaxyl as standard, 1.25%) on different fungi.



CONCLUSION

Plant extract of *Acorus calamus* L, *Nicotiana tabacum* L, *Adhatoda vasica* L, *Artemisia vulgaris* L shows significant control on cabbage aphids. *Nicotiana tabacum* L, showed prominent effect even at lowest concentration i.e. 1.25% with 50% of aphids mortality and at 20% concentration the mortality was 100%. Various plants at different concentration of extract exhibited that the plants are highly specific to the species of the fungi. The botanicals significantly reduced damage caused on plant by the cabbage aphids and fungi. These botanical powder extracts are readily available locally and environmentally friendly with low cost. Hence, there is need for farmers to adopt the use of these botanicals rather than high cost synthetic pesticides that are harmful to humans and our environment. Also possible commercial formulation for resource poor farmers is encouraged, by isolating the active constituents that are responsible for the toxicological effects of these plants.

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